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OBSERVATIONS ON INTERMEDIARY METABOLISM IN THE HUMAN FOREARM

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Thesis presented for the degree of Doctor of Medicine.

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11 September 1962

I hereby certify that the thesis entitled "Observations on Intermediary Metabolism in the Human Forearm" is my own work. It has not been presented for any degree of another university.

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December 5, 1962

Robert V. Izard
NOTARY PUBLIC.

My Commission Expires May 6, 1963

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Errata

Chapter 6, Fig. 2. For V.F. read H.K. and vice versa, and for D.C. read C.S. and vice versa.

Chapter 8, Fig. 5. The Y-axis should read from 0 to 0.18 μEq/ml, **not** from 0 to 1.8 μEq/ml.

The energy mechanisms present in skeletal muscle have been extensively studied by biochemists using in vitro system s. (For example see 1, 2, 3). By contrast, attempts to quantify skeletal muscle metabolism in situ -- and thereby to establish the pattern of energy metabolism in use, rather than that which is potentially available -- have been relatively infrequent. While the human forearm is undoubtedly composed of a number of different tissues, by far its greatest bulk is skeletal muscle. Correctly used, it provides a readily accessible tool for the appraisal of skeletal muscle metabolism in situ. The material to be presented in this dissertation describes the results of studies of basal metabolism of skeletal muscle and adipose tissue in the human forearm in control subjects and in individuals in whom metabolism was altered either by obesity or by endocrine disease.

The studies are based upon the application of, and strict adherence to the principle outline by Fick in 1870 (4).

THE FICK PRINCIPLE

During so-called "steady-state conditions", uptake (or production) of a metabolite by forearm tissues (designated $\dot{Q}$), in units of mass per minute is given by the following equation:

$$\dot{Q} = F(A - DV)$$

Where F = blood (or plasma) flow in ml per minute per unit of mass selected, and A and DV are the concentrations of metabolite in arterial and deep venous blood (or plasma) respectively in units

of mass per ml. It is essential to proper employment of the Fick principle that the following conditions obtain:

1. The arterial concentration of the metabolites under study must remain constant for the duration of the experimental procedure. Studies are therefore conducted in the post-absorptive state, that is 12-14 hours after the last meal. In the presence of either rising or falling arterial concentrations, simple measurement of arteriovenous differences will give a false reflection of metabolic exchange of the measured metabolite. Zierler (5) has recently examined this question critically and, while urging that steady state experiments be designed whenever possible, he has defined a non-steady state Fick principle which may be employed provided the transit time of the metabolite through the system is known, and provided arterial concentration is changing in linear fashion. Briefly, venous concentration should, under these circumstances, be compared not with the simultaneously measured arterial concentration, but with the arterial concentration obtaining at some earlier moment in time, this time period corresponding to the mean transit time through the system of the metabolite under study.

2. Blood flow must remain acceptably constant. During rapid changes in blood flow, calculation of $\dot{Q}$ via the Fick principle is improper, yielding values which are too large, if measured as blood flow increases, and values which are too small,

if measured as blood flow decreases. In contrast to linear alterations in arterial concentration of metabolites, it is Zierler's view that no approximation of true uptake or output is possible in the presence of rapid alterations in blood flow.

EXPERIMENTAL METHODS

Experiments were performed in an air-conditioned room, the subjects reclining comfortably on a couch, with the arm well supported. All subjects were studied during the morning hours 8 A.M. to 12 noon, some 12-16 hours after the last meal. Great care was exercised in explaining the procedure to the subjects, and there was usually little overt anxiety. An 18-gauge thin walled Riley needle was threaded upstream into the brachial artery. A 20-gauge thin walled needle was then threaded through the Riley needle, thus converting it into a double lumen needle, with the tip of the inner needle projecting 5 cm beyond the tip of the outer needle. The lumen of the 20-gauge needle was used for the collection of the arterial blood sample; continuous injection of Evans blue dye, for determination of forearm blood flow, was made through the space between the two needles. Every precaution was taken to insure that arterial samples were collected without imparting any motion to the arterial needle, which may stimulate the artery mechanically, and so alter blood flow, thereby invalidating the Fick principle (see above). Polyethylene catheters (9 inch lengths of PE 90 tubing) were inserted in retrograde

fashion ("downstream") into two forearm veins. One catheter was passed through the deep communicating vein in the antecubital fossa until its tip was no longer palpable. Blood obtained from this catheter is called "deep venous blood", and will be identified by the symbol DV. A - DV differences reflect metabolism of muscle in the main. A second catheter was placed in the largest accessible superficial vein and its tip remained palpable throughout. Blood obtained from this catheter is called superficial venous blood, and is identified by the symbol SV. A - SV concentration differences reflect metabolism of skin and subcutaneous adipose tissue in the main. The experimental technique thus allows of simultaneous sampling from brachial artery and a draining deep (muscular) vein and draining superficial (skin and subcutaneous adipose tissue) vein. In all experiments at least two sets of samples were obtained under basal conditions, and values for basal metabolism are expressed as the means thereof.

In some experiments, the technique was used to examine the local effect of various hormones, in particular insulin and human growth hormone. The hormone under study was added to the solution T-1824, which is employed for measurement of forearm blood flow, and injected intra-arterially at constant rate in amounts so small that their arrival into the general circulation stimulated no measurable response. The observed response therefore constitutes, as far as possible, the direct effects of the hormone or forearm tissues.

ANALYTICAL METHODS

It is essential that blood be handled expeditiously in these experiments. The plugged syringes were carried from the patient room to the laboratory, a few steps away, where a drop of mercury was drawn into each syringe and the blood was mixed by swirling. Blood was then distributed from the syringe into 3 tubes, two of which were in ice water, and one was at room temperature. Blood in one of the chilled tubes was immediately laked and somogyi precipitating agents were added. The supernatant was later used for glucose and lactate determinations. The second chilled tube was centrifuged in the cold and the plasma was held for FFA (free fatty acid) analysis. The third tube was centrifuged at room temperature. Potassium was determined on this plasma. Haematocrit determinations were performed on all samples to insure that there had been no saline contamination of venous samples. When gas analyses were performed, part of the blood was kept in the capped syringe which was then placed in ice water while awaiting analysis.

Blood glucose was determined by the glucose oxidase method (6); lactic acid by the lactic dehydrogenase -- DPN method (7); plasma free fatty acids by the Dole method (8); potassium using internal standard flame photometry; oxygen and carbon dioxide were determined by the method of Van Slyke and Neill (9). While these methods show essentially only minor modifications from the

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original descriptions, they have been examined extensively, and validated thoroughly in our laboratory. Dead spaces were measured for all syringes employed for collection of blood samples. Dead spaces of the collecting syringes were filled with heparin in isotonic sodium chloride.

Forearm volume was determined by water displacement of the segment between the proximal margin of the sphygmomanometer cuff at the wrist, and the antecubital crease.

DETERMINATION OF FOREARM BLOOD FLOW

Blood flow through the brachial artery was measured by the indicator-dilution technique, based on the continuous injection, at constant rate, of the dye T-1824 (Evans blue) (10). Intra-arterial injection of the dye was delivered by a power driven steel syringe, at a rate of approximately 0.1 ml per minute. Concentration of the dye in both DV and SV plasma was measured by recording optical density at 620 millimicrons on the Beckman DU spectrophotometer.

Brachial arterial plasma flow (F) is given by the equation

$$F = \frac{I}{Cm}$$

I = rate of intra-arterial injection of T-1824 in mg per minute. During steady state conditions, the rate at which the indicator leaves the local bed is also I mg per minute.

Cm = mean venous dye concentration in mg per ml. Cm is obtained from the equation

$$Cm = \frac{Cdv + Csv}{2}$$

where Cdv and Csv are the dye concentrations in Dv and Sv respectively. Measurement of blood flow by indicator dilution methods is only acceptable if the indicator mixes satisfactorily with arterial blood close to the injection site, and before any major arterial bifurcation. Arterial mixing is considered adequate if dye concentration in DV and SV plasma agree closely. If, on the other hand, the mean relative difference in dye concentration in the two veins exceeds 20 per cent, it is considered that adequate mixing of dye and blood has not occurred, and a determination of blood flow is impossible. This is, in fact, a rather rare occurrence.

Blood flow is determined from the equation

$$\text{Blood flow} = \frac{\text{Plasma flow}}{\text{Haematocrit}}$$

SHORTCOMINGS OF TECHNIQUE

The human forearm is heterogeneous. We are therefore unable to completely divorce skeletal muscle and adipose tissue from other structures in the forearm, and consequently a number of inaccuracies result:

1. Blood flow, as measured by indicator-dilution techniques, represents total flow through the brachial artery, that is, essentially total forearm blood flow. Forearm skeletal muscle blood flow is therefore overestimated. Andres, Cader and Zierler (11) after reviewing the available evidence concluded that blood flow

to muscle probably represented 80-85 per cent of total forearm flow; while blood flow through forearm bone is probably negligible, blood flow through skin is considerable, with figures in the literature suggesting that it may vary from 8 to 30 per cent of total forearm flow. The degree to which indicator dilution techniques overestimate muscle flow is thus quite variable; on the average, however, true muscle flow is about 80 per cent of total forearm flow.

2. On the other hand, uptake (and production) of metabolites by forearm muscle is expressed in units of mass per 100 ml of forearm, which therefore underestimates uptake of skeletal muscle itself. Data, based on the careful dissections of Abramson and Ferris (12), and Cooper et al (13), suggests that forearm muscle mass may vary between 50% and 75% of total forearm volume. On the average, forearm muscle occupied 58% to 66% of forearm volume; the value of $\dot{Q}$, obtained by our methods, is therefore about 60 per cent of $\dot{Q}$ of skeletal muscle proper.

3. While, in general, the metabolism of skeletal muscle is reflected by A - DV concentration differences and the metabolism of skin and adipose tissue by A - SV concentration differences, there is undoubtedly a good deal of admixture between the two veins. This is to be anticipated, partly because of the luxuriant intercommunication between deep and superficial veins, and partly because the insertion of catheters is an entirely blind procedure.

QUANTITATIVE ASPECTS OF BASAL FOREARM METABOLISM

1. Blood flow. Estimations of forearm blood flow determined by a variety of techniques show a good degree of agreement (Table I). For comparison with the data to be reported in this dissertation, particular attention will be given to the results obtained by Zierler and colleagues, whose methods the author has employed. In a recent analysis of sixty-one subjects by Zierler's group, mean forearm blood flow was found to be 3.7 ml per minute per 100 ml of arm (17).

2. Oxygen uptake ($\dot{Q}O_2$). Arteriovenous oxygen differences and oxygen uptake by forearm tissues as determined by various authors are listed in Table 2. In general, the consistency of the data is noteworthy. Mean A - DV oxygen difference of 3.02 μM/ml recorded by Zierler et al is somewhat higher than that reported by earlier workers. Collection of blood from a "deep" muscular vein, and exclusion of blood flow from the hand during periods of sampling, probably accounts for this difference.

3. Carbon dioxide production. Table 2 lists negative A - DV CO_2 differences and CO_2 production by forearm tissues appearing in the literature.

4. Glucose uptake ($\dot{Q}G$). A summary of arteriovenous glucose concentration differences, and values for glucose uptake by the forearm, as recorded by various authors, is contained in Table 3. Since the mean A - DV glucose concentration difference in Zierler's hands is only 0.14 μM/ml (or 2.5 mgm) (17), the need for great

technical accuracy is underlined. It is clear that spurious negative A - V differences may be found, particularly when arterial glucose concentrations are not stable.

5. Potassium output. The only systematic study of potassium movement across forearm muscle appears to be that of Zierler and colleagues (17, 30). Control subjects studied in the morning hours 12-16 hours after the last meal, exhibit net movement of potassium out of forearm muscle into venous plasma. During the night there appears to be no net movement of potassium between muscle and plasma. Restitution of muscle potassium clearly occurs only after partaking a meal, possibly under the influence of endogenous insulin.

6. Lactate. Contrary to the previously widespread belief that lactic acid appears in the blood only as a consequence of anoxia, Zierler et al have conclusively demonstrated the consistent production of lactate by forearm muscle under basal resting conditions, in the face of vigorous oxygen consumption (11, 17).

The fraction of glucose uptake accounted for by lactate production is given by the following equation:

$$\frac{2(DV - A)\ \text{lactate}}{(A - DV)\ \text{glucose}}$$

since production of 2 moles of lactate implies dissimilation of 1 mole of glucose. Under basal conditions, the mean value of this ratio (the L/G ratio) is 0.42 (17).

7. The possible nature of substrate(s) of forearm muscle.

The fraction of O_2 consumption accounted for by oxidation of glucose abstracted from blood is obtained from the following equation:

$$\frac{6\,[(A - DV)\ \text{glucose} - \tfrac{1}{2}(DV - A)\ \text{lactate}]}{(A - DV)\ \text{oxygen}}$$

From Zierler's data (17), it is apparent that glucose oxidation accounts for less than 20% of O_2 uptake by forearm tissues. Mean value of 0.76 for Respiratory Quotient (R.Q.) of forearm muscle suggests that the missing substrate is lipid in nature. It has been suggested that the fraction of fatty acid transported in intimate association with serum albumin, the free fatty acid (FFA) fraction, may provide the missing substrate (32). Circulating FFA turns over with extraordinary rapidity, its half life in plasma being about 2 minutes.

Over a 6 year period, Zierler's group have been unable to obtain direct evidence that FFA is the missing substrate for forearm muscle. It is possible that failure to show a consistently positive A - DV FFA concentration difference may be determined by anatomical factors. While skeletal muscle may abstract FFA from arterial plasma, adipose tissue, in close contiguity to muscle, simultaneously releases FFA into venous plasma, thereby obliterating evidence of FFA uptake by forearm tissues. A truly quantitative estimate of the role of FFA in muscle metabolism remains a challenging problem for the future.

THE HETEROGENEITY OF THE HUMAN FOREARM: METABOLISM OF THE SUPERFICIAL DRAINAGE BED

It has been pointed out that the human forearm is composed of a number of tissues other than skeletal muscle. Furthermore, the work of Mottram et al (13) indicates that about 20% of forearm blood flow passes through the skin. It is therefore not unexpected that examination of A - DV and A - SV concentration differences of a number of metabolites has revealed a patterned heterogeneity in the human forearm. While we are unable to accurately estimate either blood flow through the subcutaneous adipose tissue or the mass of this adipose tissue, useful information on adipose tissue metabolism is still obtained by comparing arterial and superficial venous concentration differences of several metabolites:

1. In blood draining superficial forearm tissues, the concentrations of glucose and potassium are lower, and the concentrations of lactate, FFA and oxygen are higher than in blood draining chiefly deep tissues of the forearm.

2. There is no significant difference between arterial and superficial venous plasma K^+ concentrations.

3. FFA are discharged from superficial tissues into the venous blood. This reflects production of FFA by subcutaneous adipose tissue, and its direct release into venous blood. Since this mechanism is under exquisitely sensitive endocrine control, it affords an excellent opportunity for the study of the action of a variety of hormones on adipose tissue in situ.

Table 4 lists the values obtained by Zierler et al in their systematic study of A - SV concentration differences of glucose, lactate, potassium and FFA (17).

THE EFFECT OF INSULIN ON FOREARM METABOLISM IN MAN

The technique employed for appraisal of forearm metabolism in man in situ lends itself nicely to the examination of local effects of hormones. The hormone, insulin in this instance, is added to the solution of blue dye used for measuring forearm blood flow, and injected by close intra-arterial injection at constant rate in amounts large enough to exert measurable local effects, but so small that arrival into the general circulation stimulates no systemic response. Thus one observes as far as possible only the effects of the hormone on forearm tissues. The response is not complicated by any counter-regulatory measures the body may institute.

In the studies of Zierler et al (33), glucagon-free insulin was injected in a dose of 100 μunits per kg. per minute for 26 minutes. When this dose is diluted by brachial arterial plasma flowing by the injector site, a concentration of insulin of approximately 300 μunits per ml is achieved in the brachial artery. The total dose of insulin was always less than 0.2 units. By contrast in the standard intravenous insulin tolerance test, the dose is about 7 units or greater.

Table 5 lists the mean response of forearm muscle to administration of insulin in this fashion to ten healthy young subjects by Zierler's group.

Intra-arterial administration of insulin had of course been frequently employed prior to Zierler's study. Improper use of the Fick principle renders much of this work uninterpretable. Most workers can be faulted for using too large a dose of insulin, rapidly administered (Table 6). By provoking systemic changes, and thereby causing rapid alterations in the arterial concentrations of the metabolites under study, the chief purpose of giving insulin via the intra-arterial route is nullified. The procedure now mimics intravenous injection, and carries similar disadvantages.

This can be avoided by choosing a sufficiently small dose and administering the insulin by constant infusion. This procedure has been faithfully followed in the studies to be reported in this dissertation.

The important observations emerging from Zierler's authoritative study on the local effects of insulin on forearm metabolism include:

1. A concentration of several hundred microunits insulin per ml brachial arterial plasma produces a tenfold or greater increase in glucose uptake by forearm muscle in control subjects.

2. Only a minor fraction of the glucose abstracted from blood under insulin stimulation is accounted for by lactate production, and none appears to be oxidised. It is probable that most of it is deposited in forearm muscle, probably as glycogen.

3. The R.Q. of forearm muscle does not rise following insulin administration.

4. Insulin provokes potassium uptake by forearm muscle, as promptly as it promotes glucose entry. The peak effect with respect to K^+ uptake is delayed, compared to that of glucose, and the decline in K^+ uptake is much slower than that of glucose uptake. Furthermore, the ratio of the insulin effect on glucose uptake to the insulin effect on K^+ uptake is inconstant. This makes untenable the classic hypothesis that movement of K^+ induced by insulin is consequent upon glucose movement.

REFERENCES

1. Cantarow, A., and Schepartz, B. Biochemistry. W. B. Saunders Company, Philadelphia, 1962.
2. Walker, B.S., Boyd, W.C., and Asimor, I. Biochemistry and Human Metabolism. Williams and Wilkins Company, Baltimore, 1957.
3. Hawk, P.B., Oser, B.L., and Summerson, W.H. Practical Physiological Chemistry. The Blakeston Company, Inc. Toronto, 1954.
4. Fick, A. Ueber die Messung des Blutquantums in den Herzventrikeln. S.-B. phys.-med. Ges. Wurzburg 1870, p. 16.
5. Zierler, K.L. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. J. Clin. Invest. 1961, 40, 2111.
6. McCann, W.P., and Jude, J.R. The synthesis of glucose by the kidney. Bull. Johns Hopkins Hosp. 1958, 103, 77.
7. Horn, H.D., and Bruns, F.H. Quantitativ bestimmung vor L(+)-Milchsaure mit milchsaure dehydrogenase. Biochem. Biophys. acta 1956, 21, 378.
8. Dole, V.P. A relation between unesterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 1956, 35, 150.
9. Van Slyke, D.D., and Neill, J.M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. Biol. Chem. 1924, 61, 523.

10. Andres, R., Zierler, K. L., Anderson, H.M., Stainsby, W.N., Cader, G., Ghrayyib, A.S., and Lilienthal, J.L., Jr. Measurements of blood flow and volume in the forearm of man; with notes on the theory of indicator dilution and on production of turbulence, hemolysis, and vasodilation by intra-vascular injection. J. Clin. Invest., 1954, 33, 482.

11. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrates in oxidative metabolism by skeletal muscle in intact man in the basal state. J. Clin. Invest., 1956, 35, 671.

12. Abramson, D.I., and Ferris, E.B. Jr. Responses of blood vessels in the resting hand and forearm to various stimuli. Am. Heart J., 1940, 19, 541.

13. Cooper, K.E., Edholm, O.G., and Mottram, R.F. The blood flow in skin and muscle of the human forearm. J. Physiol., 1955, 128, 258.

14. Barcroft, H., and Edholm, O.G. Temperature and blood flow in the human forearm. J. Physiol., 1946, 104, 366.

15. Slaughter, O.L., Brown, H.S., and Wakim, K.G. Effects of tetraethylammonium chloride on blood flow in the extremities of man. J. Lab. and Clin. Med., 1948, 33, 743.

16. Barcroft, H., and Edholm, O.G. The effect of temperature on blood flow and deep temperature in the human forearm. J. Physiol., 1943, 102, 5.

17. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. Clin. Invest., 1962, 41, 116.

18. Stadie, W.C. The oxygen of the arterial and venous blood in pneumonia and its relation to cyanosis. J. Exper. Med., 1919, 30, 215.

19. Harrop, G.A., Jr. The oxygen and carbon dioxide content of arterial and of venous blood in normal individuals and in patients with anaemia and heart disease. J. Exper. Med., 1919, 30, 241.

20. Keys, A. The oxygen saturation of the venous blood in normal human subjects. Am. J. Physiol., 1938, 124, 13.

21. Holling, H.E. Observations on the oxygen content of venous blood from the arm vein and on the oxygen consumption of resting human muscle. Clin. Sc., 1939, 4, 103.

22. Mottram, R.F. The oxygen consumption of human skeletal muscle in vivo. J. Physiol. (1955) 128, 268.

23. Mottram, R.F. The interrelationship of blood flow, oxygen utilisation of blood and oxygen consumption of human skeletal muscle. J. Physiol. (1958) 142, 314.

24. Abramson, D.I., Kahn, A., Tuck, S., Jr., Turman, G.A., Rejal, H., and Fleischer, C.J. Relationship between a range of tissue temperature and local oxygen uptake in the human forearm. I. Changes observed under resting conditions. J. Clin. Invest. (1958) 37, 1031.

25. Peters, J.P., Jr., Barr, D.P., and Rule, F.D. I. The carbon dioxide tension of the blood of normal resting individuals. J. Biol. Chem., 1948, 174, 189.

26. Somogyi, M. Studies of arteriovenous differences in blood sugar. I. Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. J. Biol. Chem., 1948, 174, 337.

28. Brown, E., and Mottram, F.R. (1959) Quoted by Mottram, R.F. and Butterfield, W.J.H. Proc. Roy. Soc. Med., 1961, 54, 149.

29. Butterfield, W.J.H., and Holling, H.E. Peripheral glucose metabolism in fasting control subjects and diabetic patients. Clin. Sci. 1959, 18, 147.

30. Andres, R., Cader, G., Goldman, P., and Zierler, K.L. Net potassium movement between resting muscle and plasma in man in the basal state and during the night. J. Clin. Invest. 1957, 36, 723.

31. Butterfield, W.J.H., and Schless, G. Observations on the peripheral metabolism of nonesterified fatty acids. Diabetes, 1959, 8, 450.

32. Fredrickson, D.S., and Gordon, R.S., Jr. The metabolism of albumin-bound C^{14}-labeled unesterified fatty acids in normal human subjects. J. Clin. Invest., 1958, 37, 1504.

33. Andres, R., Baltzan, M.A., Cader, G., and Zierler, K.L. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J. Clin. Invest. 1962, 41, 108.

34. Bell, D.M., and Burns, T. Effects on femoral A-V glucose difference of insulin injected into an antecubital vein and into a femoral artery. J. Clin. Invest., 1952, 31, 717.

35. Hardwick, C., Butterfield, W.J.H., Fry, I.K., Briggs, J.H. Direct measurement of the effect of insulin on the uptake of glucose by peripheral muscles in normal subjects, diabetics and acromegalics. Proc. Roy. Soc. Med., 1959, 52, 807.

36. Ginsberg, J., Galbraith, H.J.B., and Paton, A. Some peripheral effects of insulin and other hormones in man. The Mechanism of Action of Insulin. Edited by Broom, W.A., and Wolff, F.W. p. 225, Blackwell, 1960.

Table I

Observations on Forearm Blood Flow

Author	Water Bath Temperature	Ambient Temperature	Blood Flow ml/min/100 ml forearm Mean	Range	Method	Reference
Barcroft and Edholm		18.5 C	3.1	2.6 -3.6	Air plethysmograph	14
Slaughter et al.		27-29 C	4.9	1.7 -7.3	Air plethysmograph	15
Barcroft and Edholm	33 C	15-20 C	2.7	1.8 -3.8	Water plethysmograph	14, 16
Andres et al.		25-27 C	4.7	2.7 -7.1	Dye dilution	10
Baltzan et al.		25-27 C	3.71	±0.173*	Dye dilution	17

*Data presented as means ±S.E. of mean.

Table II

Observations on Forearm Gas Exchange

Author	A-V Difference mM/l	Uptake μM/min/100 ml arm	Reference
Oxygen			
Stadie (1919)	2.14		18
Harrop (1919)	2.44		19
Keys (1938)	2.53		20
Holling (1939)		8.5	21
Mottram (1955)		10.7	22
Andres et al. (1956)	3.24±0.323*	11.7±1.34*	11
Mottram (1958)		12.0	23
Abramson et al. (1958)	3.4	9.4	24
Baltzan et al. (1962)	3.02±0.168*	11.6±0.968*	17
Carbon dioxide			
Harrop (1919)	-2.23		19
Peters et al. (1920)	-3.03		25
Andres et al. (1956)	-2.5 ±0.2*	-8.7±0.94*	11

*Values expressed as means ±S.E. of mean.

Table III

Observations on Forearm Metabolism

Metabolite	Author	A-V Difference mM/l	Forearm Uptake μM/min/ 100 ml arm	Reference	Remarks
Glucose	Somogyi (1948)	0.275		26	
	Bell (1952)	0.12		27	Femoral A-V concentration differences
	Andres et al. (1956)	0.093±0.029	0.38±0.143	11	
	Brown and Mottram (1959)		0.82	28	
	Butterfield and Holling (1959)	0.147	0.44	29	
	Baltzan et al. (1962)	0.14 ±0.017	0.52±0.075	17	

Table III (continued)

Observations on Forearm Metabolism

Metabolite	Author	A-V Difference mM/l	Forearm Uptake μM/min/ 100 ml arm	Reference	Remarks
Lactate	Andres et al. (1956)	-0.11±0.022	-0.42±0.116	11	
	Baltzan et al. (1962)	-0.12±0.012	-0.44±0.060	17	
Potassium	Andres et al. (1957)	-0.23±0.055	-0.66±0.233	30	
	Baltzan et al. (1962)	-0.19±0.027	-0.43±0.076	17	
FFA	Butterfield and Schless	+0.52	+3.6	31	Extremely wide range in arterial FFA. Mean uptake sufficient to account for 6 times forearm Q_{O_2}
	Baltzan et al.	+0.02±0.022		17	Mean uptake of FFA by forearm apparently zero

When applicable values are means ±S.E. of mean.

Table IV

Arterio Superficial Venous Concentration Differences

Metabolite	(A-SV) Concentration Difference mM/l
Glucose	+0.19 ± 0.027
Potassium	-0.03 ± 0.021
Lactate	-0.18 ± 0.015
FFA	-0.13 ± 0.028

Data from Baltzan, Andres, Cader and Zierler (17).

All values are means ±S.E.M.

Table V

Mean Effect of Insulin on Forearm Metabolism

Time after insulin started min	Plasma Flow	Glucose A-DV	Glucose $\dot{Q}$	Lactate A-DV	Potassium A-DV	Potassium $\dot{Q}$
0	2.1±0.14	0.16±0.038	0.59±0.178	-0.14±0.026	-0.29±0.093	-0.65±0.255
12	2.7±0.32	0.60±0.150	2.70±0.811	-0.16±0.035	-0.01±0.066	-0.03±0.242
18	2.6±0.26	1.11±0.185	4.71±1.026	-0.16±0.026	+0.12±0.037	+0.41±0.216
26	3.1±0.28	1.29±0.145	6.78±1.250	-0.22±0.034	+0.17±0.047	+0.59±0.153
40	2.7±0.24	1.36±0.165	6.07±0.998	-0.24±0.042	+0.35±0.034	+0.95±0.161
60	2.6±0.32	0.86±0.176	3.52±0.645	-0.24±0.340	+0.32±0.067	+0.79±0.221
90	2.9±0.42	0.55±0.147	2.41±0.527	-0.22±0.040	+0.23±0.024	+0.63±0.163

Data from Andres, Baltzan, Cader and Zierler (33).

Insulin administered intra-arterially for 26 minutes beginning at time zero. Values are means ±S.E. of means among ten young subjects of normal body weight.

Table VI

Effects of Intra-arterial Insulin on Forearm Glucose Uptake

Authors	References	Concentration of Insulin Injected	Maximal Effect on Glucose (A-V) μM/ml	Maximal Effect on Glucose $\dot{Q}$ μM/min/100 ml arm	Remarks
Bell and Burns	34	0.0125 U/kg into the femoral artery over 1/2 minute period	0.6		17 per cent in arterial glucose contration
Butterfield et al.	35	0.1 U injected into brachial artery over 2 minute period		3.0	Wide variations in blood flow reported. Arterial concentrations reported as constant
Ginsberg et al.	36	2 U injected rapidly into brachial artery	1.65		30 per cent fall in mean arterial concentration

CHAPTER II

ROLE OF FREE FATTY ACIDS IN FOREARM METABOLISM IN MAN, QUANTITATED BY USE OF INSULIN

Previous studies (1, 2) have demonstrated that glucose uptake by muscle of the forearm of man is inadequate to account for more than about 10 per cent of its observed oxygen consumption. Because forearm respiratory quotient (RQ) is approximately 0.7, which suggests combustion of long-chain fatty acids, it was predicted that the fraction of plasma lipid known as free fatty acids (FFA) would prove to be the missing substrate of forearm muscle. Extensive examination of this problem has, however, failed to demonstrate consistently positive arterio-deep venous (A - DV) FFA differences across the human forearm (2). There is evidence, based on isotopic studies by Friedberg, Klein, Trout, Bogdonoff, and Estes (3), that the human forearm does remove FFA from arterial blood, and the possible factors responsible for our failure to demonstrate this by simple measurement of A - DV concentration differences have been treated in detail elsewhere (2). In brief,while FFA is being extracted from plasma by forearm muscle, adipose tissue, intimately surrounding muscle, adds fatty acid to venous plasma. The net A - DV FFA difference is determined by the relative rates of these two processes.

The validity of this hypothesis could be tested if there were an agent that inhibited release of FFA from adipose tissue without also affecting FFA uptake by peripheral tissues. Insulin may be such an agent.

There is no doubt that insulin inhibits FFA release from adipose tissue, a fact demonstrated both *in vivo* by Gordon (4), Estes and associates (5), and Bierman, Schwartz, and Dole (6), and *in vitro* (7, 8). There is, however, conflicting evidence on whether or not insulin influences FFA uptake by peripheral tissues. In excised adipose tissue, in the presence of both glucose and insulin, FFA incorporation is increased (7). But there is no such evidence from experiments *in vivo*. Indeed, it is unproven that there is substantial FFA uptake by adipose tissue *in vivo* under any circumstances. Bragdon and Gordon (9) found only 1 to 2 per cent of injected palmitic acid-1-C^{14} in adipose tissue, a figure not increased by prior administration of glucose, which stimulates insulin secretion. It has been demonstrated by Spitzer and Hohenleitner in the dog (10) and by Scow, Robert, and Chernick (11) in isolated rat parametrial adipose tissue that, while net production of FFA by adipose tissue was inhibited by insulin, significant net uptake by adipose tissue was not produced. With respect to a possible effect of insulin on FFA uptake by muscle, experiments capable of testing this crucially *in vivo* have not been done. Absence of any such action, however, is suggested by the fact that turnover rates of FFA, measured by use of isotopes in the dog, are not changed by insulin (6, 12). Fritz (13) showed that oxidation of palmitic acid-1-C^{14} by excised rat diaphragm was not altered by insulin.

Thus, while it is not established that insulin may not influence FFA uptake by peripheral tissues, most of the available data suggest that it does not do so in the intact animal. In the light of previous studies showing that the human forearm is remarkably sensitive to local intra-arterial infusion of small concentrations of insulin (14), a technique was available to establish the effects of insulin, injected intra-arterially at a final concentration of several hundred microunits per milliliter of brachial arterial plasma, on FFA metabolism in the human forearm. Information was sought concerning the following two problems. 1) Basal arterio-superficial venous (A - SV) FFA concentration differences are almost invariably negative, thereby reflecting discharge of fatty acids from forearm adipose tissue into venous plasma. Insofar as A - SV concentration differences reflect metabolism of forearm adipose tissue, does insulin, under the conditions to be employed, in fact obliterate negative A - SV FFA concentration differences? 2) What effect, if any, does insulin exert on A - DV FFA concentration differences and, if one assumes the complete oxidation of abstracted fatty acids, is sufficient FFA uptake unmasked to account for most of the oxygen consumption of skeletal muscle? Of course glucose uptake under maximal insulin stimulation is sufficient to account entirely for oxygen consumption by forearm muscle if all the glucose is oxidized. Previous studies, however, suggest that none of the glucose entering muscle under the influence of insulin is oxidized during the time of the acute experiment (14) A preliminary report of some of these observations has appeared (15).

METHODS

Five young adult subjects were studied. Dietary history of carbohydrate intake was normal. No food was taken after 8 p.m. on the evening preceding the experiment. Studies were performed between the hours of 9 a.m. and 1 p.m. A brachial artery, an ipsilateral antecubital vein, draining muscle but also undoubtedly contiguous adipose tissue, and a superficial vein, draining mostly but not exclusively forearm adipose tissue and skin, were cannulated by techniques described previously (2). Blood flow was measured by the indicator-dilution method, based on continuous injection at constant rate of the dye T-1824 (1). Two simultaneous sets of arterial, deep venous (DV), and superficial venous (SV) samples were collected at 15-minute intervals before the infusion of insulin.

Glucagon-free insulin[1] was then injected intra-arterially for 26 minutes at a rate delivering 100 μU of insulin per minute per kg of body weight. Blood samples were drawn 12, 18, 26 minutes from commencement of the infusion, and for periods up to an hour thereafter. Circulation to the wrist and hand was excluded for at least 5 minutes preceding collection of blood samples and for the entire period of insulin administration.

Net uptake or output of a metabolite by forearm muscle was calculated from the equation $\dot{Q} = F(A - DV)$, where $\dot{Q}$ is uptake or output of the metabolite in units of mass per minute per 100 ml of forearm flow, F is blood or plasma flow through the forearm in

1 Lot no. W 3606, Eli Lilly and Company, Indianapolis, Ind.

milliliters per minute per 100 ml of arm, and A and DV are concentrations of the metabolite in arterial and deep venous blood or plasma, respectively, in units of mass per milliliter. This equation overestimates $\dot{Q}$, since it assigns all of forearm blood flow to muscle. The errors have been discussed in detail elsewhere (1). Handling of blood and analytical methods were as previously described (2).

Insulin concentration in forearm plasma. Calculated brachial arterial insulin concentration varied from 300 to 700 μU per ml, levels comparable to those achieved in an earlier study (14).

Plasma flow. Except for one subject, L, plasma flow remained acceptably constant; this is, it did not vary by more than 20 per cent about the mean in any of the remaining four subjects.

Arterial concentrations. Arterial concentrations of glucose and lactate remained constant. We have previously shown that the arterial concentration of FFA is labile under basal postabsorptive conditions (2). Changes in arterial concentration of FFA appear to be related to the height of the initial values: low concentrations tend to rise and high ones to fall. In the light of these considerations, the course of arterial concentration of FFA in the present series may be considered not to have varied unusually (Figure 1). In particular, no consistent arterial hypolipacidemia was noted after insulin administration. In one subject, L, there was a 50 per cent rise in arterial FFA just before conclusion of the study.

Glucose (Figure 2). Insulin promptly increased both A - DV and A - SV glucose concentration differences, the former ten and the latter five times the resting value. Temporal events in the deep and the superficial forearm beds paralleled each other closely, with the maximal effect reaching a plateau from approximately 18 to 40 minutes and then decaying fairly rapidly.

Lactate. A small but definite increase in negative lactate A - DV and A - SV concentration differences occurred during and after insulin administration. Because glucose uptake increased more than lactate production, the fraction of glucose uptake accounted for by lactate production (L/G ratio) decreased from a basal value of greater than 0.4 to less than 0.2 in both forearm beds after insulin administration.

A - SV FFA differences (Figure 3). Under basal conditions, negative A - SV FFA differences were present in all five subjects, reflecting discharge of fatty acids from forearm adipose tissue into venous plasma. In each of the five subjects, negative A - SV FFA differences were reduced 12 minutes after start of the insulin infusion. Samples taken 18, 26, and 40 minutes from the beginning of insulin infusion showed complete inhibition of FFA release from the superficial bed. Thereafter, the effect decayed rapidly and negative A - SV FFA differences were restored by the 90-minute sample. Since blood flow through the superficial bed constitutes only about 10 per cent of forearm blood flow (1), the positive A - SV FFA difference uncovered at peak insulin effect is quantitatively small

and probably represents uptake of FFA by nonadipose tissue drained by the superficial venous effluent, principally skin.

A - DV FFA differences (Figure 4). While values for basal A - DV FFA concentration differences showed wide variation, the mean value did not differ significantly from zero, in agreement with the larger body of evidence collected from this laboratory (2). After insulin administration, A - DV FFA differences became positive or more positive in all subjects. The magnitude of the response was related to the value of the basal A - DV difference; the largest increase occurred in the one subject, E, with a negative basal A - DV difference, indicating considerable admixture of DV by effluent from adipose tissue. Subjects exhibiting significantly positive basal A - DV differences in the neighborhood of 0.1 μmole per ml, and therefore, by inference, little contamination of DV by effluent from adipose tissue, showed only small increases with insulin. In all subjects, a positive A - DV difference of 0.07 μmole per ml or greater was uncovered under maximal insulin stimulation. The mean maximal A - DV difference achieved was 0.09 μmole per ml.

$\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, and RQ. No significant changes in O_2 consumption, CO_2 production, or RQ were observed after insulin administration. This confirms earlier observations from this laboratory (14).

G - L/O_2. If glucose removed from arterial blood by forearm tissues but not accounted for by lactate production (G - L) were all oxidized, the fraction of observed O_2 consumption accounted for by glucose oxidation, G - L/O_2, was well above unity during insulin infusion;

that is, more than enough glucose was abstracted by muscle to account for all its O_2 consumption. Evidence pointing to most of the incremental glucose being stored rather than oxidized under these conditions has been presented in detail elsewhere (14). In brief, failure of either $\dot{Q}_{O_2}$ or RQ to rise under insulin stimulation favors the notion that the glucose taken up by muscle is diverted toward glycogen synthesis. Calculation of G - L/O_2 ratio under these conditions is therefore meaningless.

FFA/O_2. Since 1 mole of FFA requires 25 moles of oxygen for complete oxidation, the fraction of O_2 uptake accounted for by oxidation of FFA abstracted from plasma, FFA/O_2, can be calculated from the following equation: FFA/O_2 = [(A - DV) FFA x 25 x plasmacrit] / $(A - DV)_{O_2}$. FFA/O_2 increased from an apparent mean basal ratio of 0.12 to a value of 0.42 or greater in each of the five subjects (Table II); that is, enough FFA was abstracted by muscle to account for about half its oxygen consumption under maximal insulin stimulation. Subjects L and S, with basal FFA/O_2 in the neighborhood of 0.5, suggesting only slight adipose tissue contamination of DV effluent, showed, as anticipated, little or no increase in FFA/O_2 with insulin; conversely, Subjects B, Y, and E, with either low or negative FFA/O_2, suggesting appreciable adipose tissue contamination of DV effluent, showed, again as anticipated, considerable increase in FFA/O_2 with insulin.

DISCUSSION

There have been a number of reports on effects of insulin on plasma FFA concentrations and on arteriovenous FFA concentration differences (4, 5, 16, 17). In most instances insulin was given intravenously, but even when administered intra-arterially, the dose was usually so large,--usually 1 U or greater--that the effect was like that of an intravenous dose. The concentration of insulin in recirculating blood affecting total body response was generally as great as that affecting the forearm alone in the present study and, by provoking rapid changes in arterial concentration of glucose and FFA, made quantification of insulin action difficult.

Insulin-induced inhibition of FFA release has, however, been unequivocally shown across the femoral arterial-saphenous venous drainage bed (4, 5) and, insofar as A - SV concentration differences reflect metabolism of forearm adipose tissue, appears to be confirmed by our own data. At the same time, in each of the five subjects a positive A - DV FFA concentration difference of 0.07 μmole per ml or greater was unmasked, although insulin appears to be without direct influence on FFA uptake by peripheral tissues. The increase in A - DV FFA and FFA/O_2 varied inversely with the magnitude of basal values for these indices. This is consistent with the notion that insulin-induced positive A - DV FFA concentration difference results from inhibition of FFA release from forearm adipose tissue, since the largest insulin effect is seen in the subjects with the largest adipose tissue contamination of DV effluent and hence low

or negative A - DV FFA and FFA/O_2. Insulin, therefore, merely unmasks FFA uptake by forearm muscle. In the light of these observations, the significance of an FFA/O_2 ratio of 0.5 under maximal insulin stimulation is that it implies that this may also be the amount of $\dot{Q}_{O_2}$ accounted for by FFA oxidation in the basal state.

Uptake of FFA by the forearm at peak response to insulin is 0.27 μmole FFA per minute per 100 ml forearm, or roughly 0.36 μmole FFA per minute per 100 g forearm skeletal muscle. FFA uptake by total skeletal muscle, which comprises 40 per cent of body weight, or approximately 25 kg, is thus approximately 0.1 mmole per minute. This figure corresponds very closely to the estimate of basal extrahepatic FFA oxidation given by Fritz in his comprehensive review of long-chain fatty acid oxidation (18).

Why FFA/O_2 ratio, even under maximal insulin stimulation, is closer to 0.5 than to the predicted 0.8 to 0.9 continues to pose a problem. One possibility arises from the suggestion that insulin may decrease the uptake of FFA by peripheral tissues (4). These observations, however, were made after glucose plus insulin administration in doses sufficient to cause a fall of 50 per cent or more in arterial FFA levels; under nonsteady-state conditions, A - V concentration differences of zero do not necessarily imply absence of net movement of metabolites. (19).

A second explanation for FFA/O_2 being in the neighborhood of 0.5 is the possibility that the missing fraction of 0.4 is provided by the diffusion of FFA from deep-lying adipose tissue into contiguous muscle through the interstitial fluid without traversing any vascular

bed. These adipose tissue depots are probably replenished in the hours immediately after the ingestion of a mixed meal. Studies in the rat, based on distribution of injected, isotopically labeled chylomicron particles, support the notion that a significant percentage is deposited between or in fibers of skeletal muscle (9).

SUMMARY

The quantitative role of free fatty acids (FFA) in metabolism of forearm muscle in man was assessed by intra-arterial administration of insulin to five healthy young men: insulin-induced inhibition of FFA release from forearm adipose tissue unmasks an uptake of FFA by forearm muscle sufficient to account for about 50 per cent of its oxygen consumption. If insulin is without direct influence on the net uptake of FFA by muscle and adipose tissue, then this figure represents the quantitative role of plasma FFA in forearm metabolism under basal conditions as well.

REFERENCES

1. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.
2. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.
3. Friedberg, S.J., Klein, R.F., Trout, D.L., Bogdonoff, M.D., and Estes, E.H., Jr. The characteristics of the peripheral transport of C^{14}-labeled palmitic acid. J. clin. Invest. 1960, 39, 1511.
4. Gordon, R.S., Jr. Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J. clin. Invest. 1957, 36, 810.
5. Estes, E.H., Jr., Bogdonoff, M.D., Friedberg, S.J., Harlan, W.R., Jr. and Trout, D.L. The effect of insulin on nonesterified fatty acid release from the human leg. J. clin. Invest. 1959, 38, 2131.
6. Bierman, E.L., Schwartz, I.L., and Dole, V.P. Action of insulin on release of fatty acids from tissue stores. Amer. J. Physiol. 1957, 191, 359.
7. Bally, P.R., Cahill, G.F., Jr., Leboeuf, B., and Renold, A.E.. Studies on rat adipose tissue *in vitro*. V. Effects of glucose and insulin on the metabolism of palmitate-1-C^{14}. J. biol. Chem. 1960, 235, 333.

8. Gordon, R.S., Jr., and Cherkes, A. Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro*. Proc. Soc. exp. Biol. (N.Y.) 1958, 97, 150.

9. Bragdon, J.H., and Gordon, R.S., Jr. Tissue distribution of C^{14} after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. J. clin. Invest. 1958, 37, 574.

10. Spitzer, J.J., and Hohenleitner, F.J. Release of free fatty acids by adipose tissue *in vivo*. J. Lipid Res. 1961, 2, 396.

11. Scow, R.O., Robert, A., and Chernick, S.S. Perfusion of adipose tissue. Fed. Proc. 1962, 21, 201.

12. Armstrong, D.T., Steele, R., Altszuler, N., Dunn, A., Bishop, J.S., and de Bodo, R.C. Plasma free fatty acid turnover during insulin-induced hypoglycemia. Amer. J. Physiol. 1961, 201, 535.

13. Fritz, I.B. Effects of insulin on glucose and palmitate metabolism by resting and stimulated rat diaphragms. Amer. J. Physiol. 1960, 198, 807.

14. Andres, R., Baltzan, M.A., Cader, G., and Zierler, K.L. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J. clin. Invest. 1962, 41, 108.

15. Rabinowitz, D., and Zierler, K.L. Effect of insulin on free fatty acid metabolism in the human forearm. Fed. Proc. 1962, 21, 200.

16. Dole, V.P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. clin. Invest. 1956, 35, 150.

17. Butterfield, W.J.H., and Schless, G. Observations on the peripheral metabolism of nonesterified fatty acids. Diabetes 1959, 8, 450.

18. Fritz, I.B. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev. 1961, 41, 52.

19. Zierler, K.L. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. J. clin. Invest. 1961, 40, 2111.

Table I

Mean Effect of Insulin on Forearm Metabolism*

After Insulin Started min	Plasma Flow ml/min/ 100 ml arm	Glucose μM/ml A-DV	Glucose μM/ml A-SV	Lactate μM/ml A-DV	Lactate μM/ml A-SV	FFA μM/ml A-DV	FFA μM/ml A-SV
0	2.0±0.18	0.12±0.016	0.17±0.013	-0.09±0.010	-0.15±0.015	+0.02±0.038	-0.16±0.033
12	2.3±0.22	0.48±0.079	0.43±0.048	-0.11±0.010	-0.12±0.011	+0.02±0.022	+0.02±0.054
18	2.4±0.17	0.88±0.086	0.65±0.061	-0.14±0.020	-0.17±0.029	+0.06±0.021	+0.06±0.016
26	2.8±0.79	1.39±0.128	0.83±0.038	-0.18±0.010	-0.23±0.030	+0.08±0.014	+0.06±0.020
40	2.3±0.29	1.28±0.060	0.80±0.032	-0.22±0.010	-0.21±0.035	+0.09±0.033	+0.09±0.023
60	2.0±0.40	0.65±0.084	0.50±0.044	-0.21±0.020	-0.24±0.037	+0.02±0.041	-0.03±0.013
90	1.7±0.25	0.54±0.138	0.47±0.057	-0.20±0.030	-0.26±0.041	-0.07±0.051	-0.06±0.042

*Insulin administered intra-arterially for 26 minutes beginning at time zero. Values given at time zero are means of all control measurements taken before insulin infusion started. Data for A-DV and A-SV concentration differences are means of values obtained in 5 subjects except for A-SV concentration differences obtained at 12 minutes, which are means of values in 4 subjects, and A-DV and A-SV concentration differences obtained at 90 minutes, which are means of values in 3 subjects. All values are means ±S.E. of means.

Table II

Effect of Insulin on R.Q., $\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$ and FFA/O_2 of Forearm Muscle

	Basal	Peak Insulin Effect
$\dot{Q}_{O_2}$ μM/min/100 ml arm	9.36 ± 1.27	9.2 ± 1.15
$\dot{Q}_{CO_2}$ μM/min/100 ml arm	6.32 ± 1.02	6.27 ± 0.78
R.Q.	0.70 ± 0.04	0.70 ± 0.06
FFA/O_2		
Subject S	0.65	0.65
L	0.48	0.60
B	0.17	0.62
Y	0.09	0.42
E	-0.77	0.69
Mean	0.12 ± 0.24	0.56 ± 0.09

FFA/O_2 = fraction of oxygen consumption accounted for by FFA oxidation. For calculation see text. Each of the 5 subjects is identified by an initial. Other values are means ±S.E. of means.

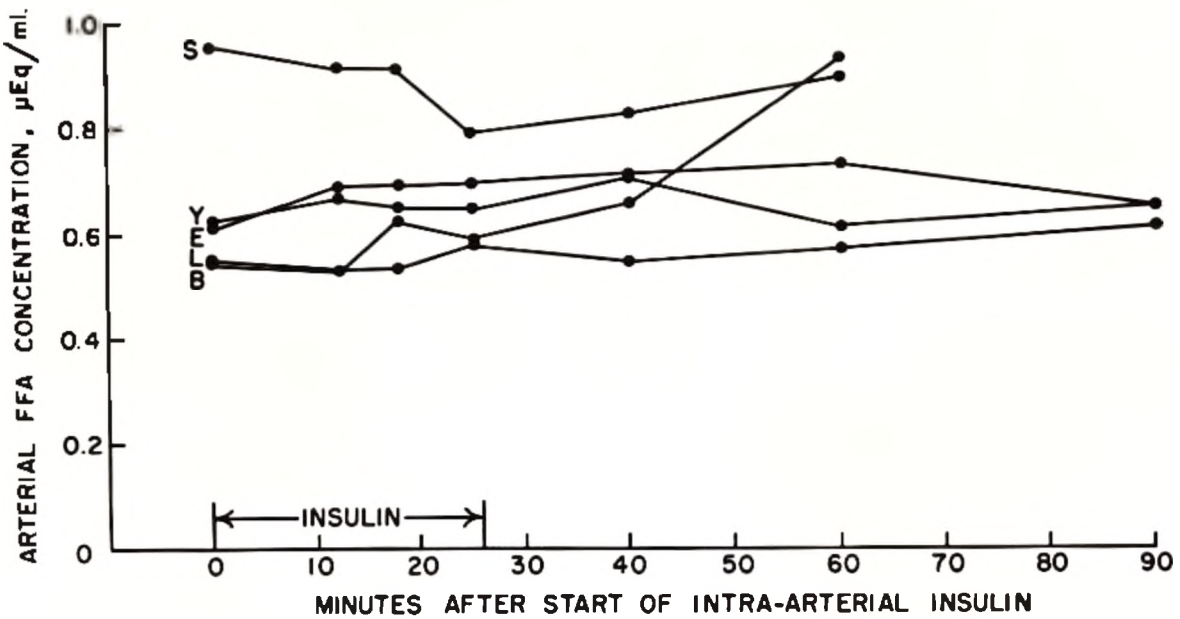

ARTERIAL FFA CONCENTRATION, μEq/ml.
1.0
0.8
0.6
0.4
0.2
0
S
Y
E
L
B
INSULIN
0
10
20
30
40
50
60
70
80
90
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 1.

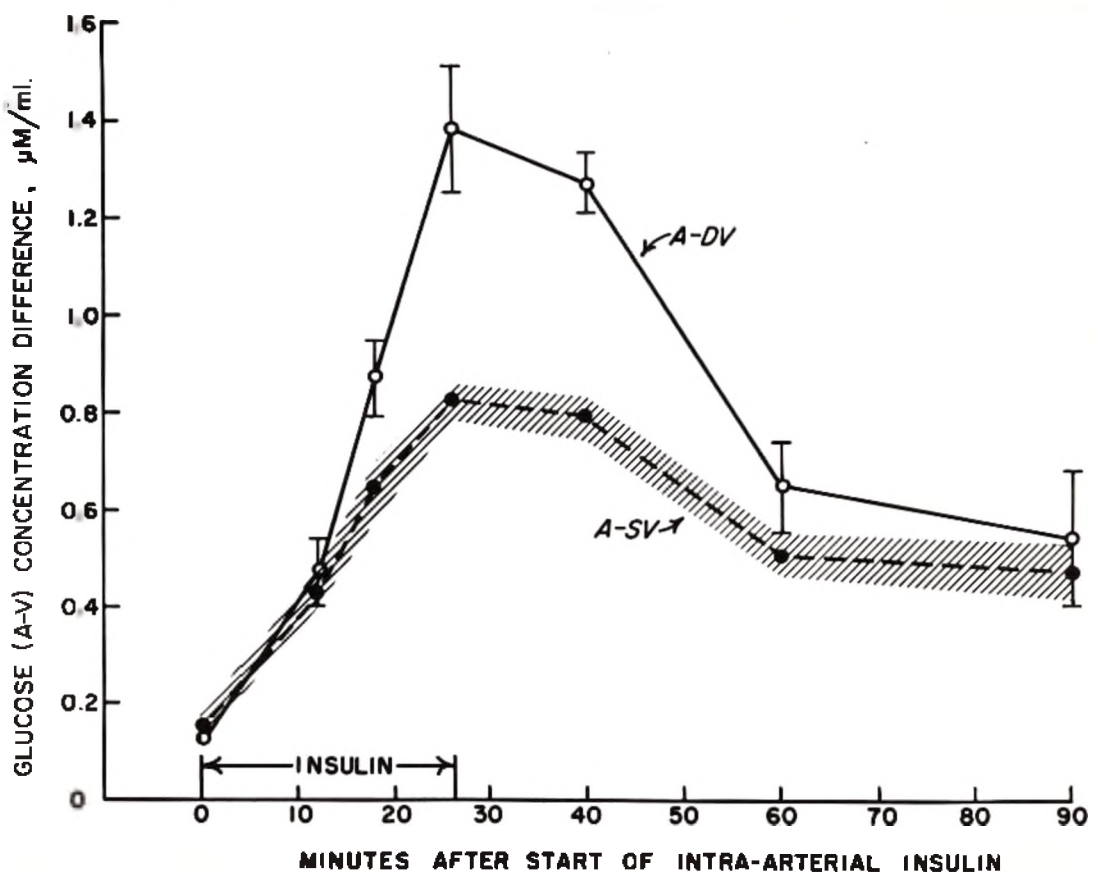

GLUCOSE (A-V) CONCENTRATION DIFFERENCE, μM/ml.
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
A-DV
A-SV
INSULIN
0
10
20
30
40
50
60
70
80
90
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 2.

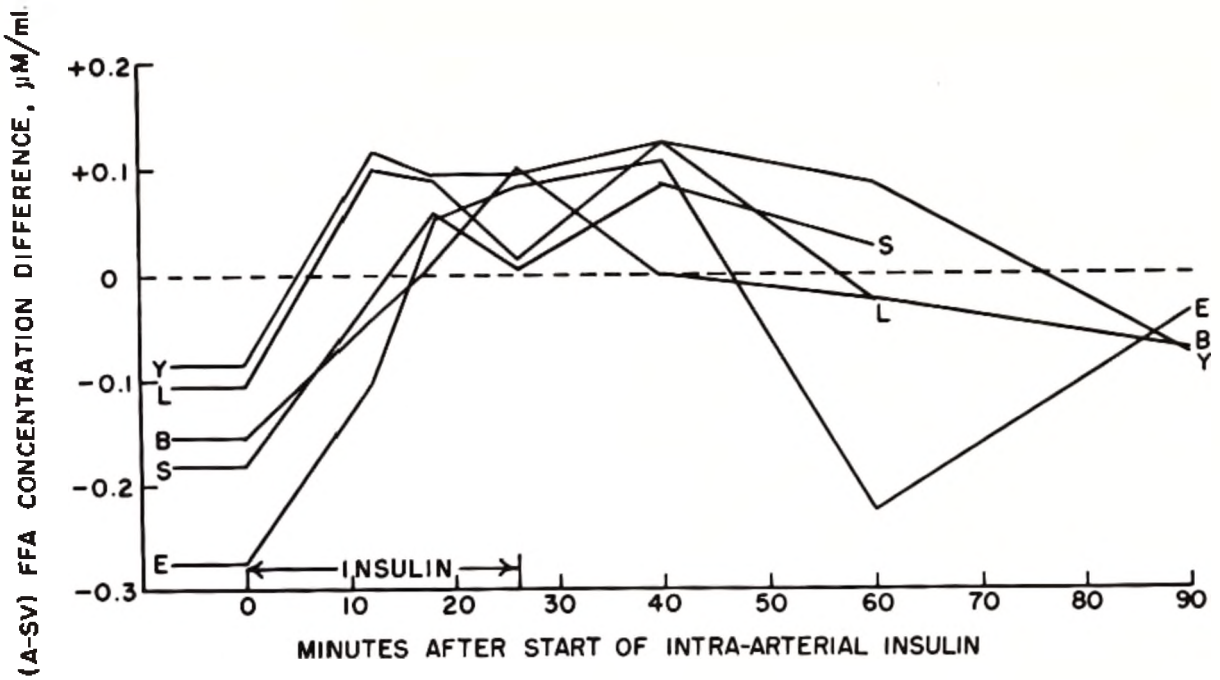

(A-SV) FFA CONCENTRATION DIFFERENCE, μM/ml.
+0.2
+0.1
0
-0.1
-0.2
-0.3
Y
L
B
S
E
S
L
E
B
Y
INSULIN
0
10
20
30
40
50
60
70
80
90
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 3

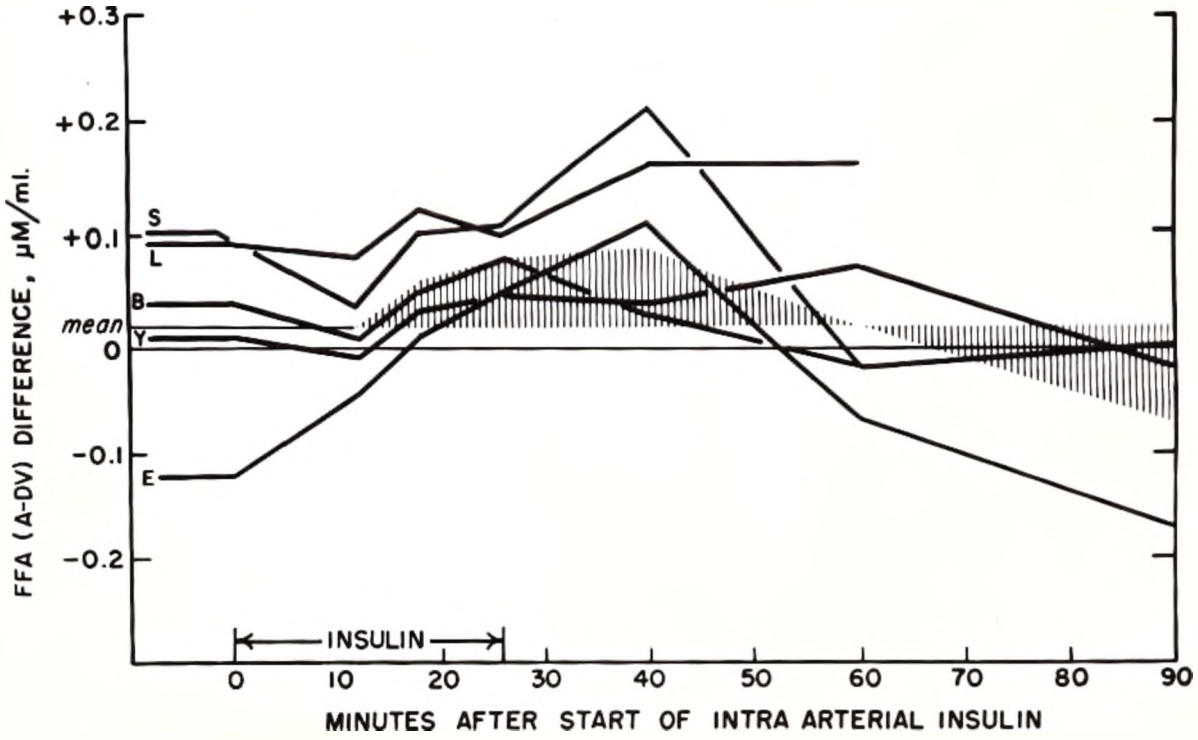

FFA (A-DV) DIFFERENCE, μM/ml.
+0.3
+0.2
+0.1
mean
0
-0.1
-0.2
S
L
B
Y
E
INSULIN
0
10
20
30
40
50
60
70
80
90
MINUTES AFTER START OF INTRA ARTERIAL INSULIN

FIG. 4.

CHAPTER III

THE AUGMENTED INSULIN TOLERANCE TEST FOR DETECTING INSULIN RESISTANCE

Acromegaly and Cushing's syndrome have long been known to be characterised by insulin resistance (Fraser, 1960), a feature usually demonstrable by the standard insulin tolerance test (I.T.T.) as originally introduced to demonstrate insulin sensitivity in panhypopituitarism (Fraser et al., 1941). The subsequent refinement of other tests such as thyroid function tests, urinary gonadotrophin measurements and the water loading test, has largely superseded the use of I.T.T. in the diagnosis of panhypopituitarism, while steroid measurements are now used to delineate the metabolic abnormality of Cushing's syndrome. We do, however, still lack suitable methods for the clinical demonstration of growth hormone overproduction. Immunoassay of serum growth hormone levels may ultimately be of diagnostic value (Read and Bryan, 1960), once the serious practical difficulties of the test have been overcome (Ciba Colloquium, 1961). In the meantime, the need for an objective measure of "activity" in acromegaly has been increased by recent developments in the treatment of this disease (Joplin et al., 1961). In this paper we report the results obtained with an augmented insulin tolerance test, using 11.1 units per square metre of body surface (three times the standard dose). The normal response to this test has been defined. Results obtained in "simple" obese subjects and in groups of acromegalics of different clinical "activity" suggested that the test is useful for the assessment of acromegaly and its response to treatment, provided that such cases have a normal fasting blood sugar and are not grossly obese.

METHODS

1. Subjects tested.

(a) Normal subjects. Nineteen subjects were tested, being volunteers from the medical staff, technicians and ambulant patients hospitalised for non-endocrine and non-metabolic diseases. Most subjects were in their twenties or early thirties, the age range being nineteen to fifty-four. There were twelve males and seven females. All were judged to be within 10 lbs. of their ideal body weight.

(b) "Untreated" acromegalics. This group comprised twelve non-diabetic euthyroid subjects with obvious acromegaly, either untreated or previously incompletely treated. Of the six subjects previously treated by deep x-ray therapy, all still had headache, paraesthesiae or other features of acromegaly which called for further treatment; the dosage of this previous therapy varied widely. These tests were done preceding needle implantation of the pituitary with radioactive seeds of Au-198 or Y-90 (Fraser and Joplin, 1961). Only one patient (C.S.) was grossly obese.

(c) Au-198 or Y-90-treated acromegaly. Thirteen subjects (14 tests) were tested following pituitary implantation with Au-198 or Y-90; five of the subjects were considered to be adequately treated, and nine others were not in complete remission (for criteria and these methods of treatment, see Joplin et al., 1961).

(d) "Simple" or uncomplicated obesity.

These were twelve subjects with gross obesity (i.e., at least 25% overweight in terms of their clinically estimated ideal weight), but otherwise healthy. The heaviest was M.Me., whose height was 5'3" and weight 19 stone. Four were male and eight female, most being in the late teens and early twenties, the ages being from thirteen to forty-nine years. All were chosen at random from the large numbers referred to the hospital for weight reduction, after clinical examination and other tests had excluded subjects with Cushing's syndrome or other associated disease. None had a known family history of diabetes mellitus.

2. Standard test procedure.

For the three days preceding the test, the subjects received a diet containing 300 G carbohydrate. Freedom from other intercurrent conditions was checked, and then the subject fasted from 8 p.m. the previous evening. The tests were performed in hospital between 8 a.m. and 11 a.m., i.e., from the twelfth to fourteenth post-absorptive hours, with the subject secluded and reclining on a couch or bed. After control blood samples, taken for capillary blood sugar (obtained by making a small stab incision with a B-P blade into a digital pad), soluble insulin was given intravenously in a dose of 11.1 units per square meter of body surface. Subsequently, capillary blood sugars were taken half-hourly for two hours with additional samples also at twenty and forty minutes. In all cases

duplicate specimens were obtained for the fasting, 60, 90 and 120 minute levels. The normal subjects experienced hypoglycemic symptoms over the twenty to sixty minute period. All subjects were questioned at each blood sugar sampling to check that speech was normal and no shock present. Immediately after the tests, a meal was given and the subsequent meals at four-hourly intervals throughout this day had to be completed. No untoward symptoms after conclusion of the tests were noted by any subjects.

RESULTS

1. Response in normal subjects.

Figure 1 shows the range of the blood sugar curve in mg% (absolute values) during this augmented test (11.1 units per square meter of body surface) in comparison with the mean curve of the standard I.T.T. (giving one-third of this dose) expressed as % of fasting level (F.L.). The initial fall or first phase, was similar after both doses, usually reaching a trough at twenty or thirty minutes of under 50% of the fasting blood sugar level. However, the subsequent curve was different; that after the standard dose immediately rising towards the fasting level and passing it before 120 minutes, while that of the augmented dose rose only slightly during this second phase from 30 to 120 minutes.

A good summary of the measure of insulin resistance by the test, was found to be the sum of the 60, 90 and 120 minute blood sugar values as mg/100 ml. Normal values of this "insulin resistance index" were mean 92, range 62-142, mean $\pm 2SD$ = 48-136.

2. "Untreated" acromegalics. (Fig. 2)

The characteristic abnormalities of the acromegalic curve were the slow initial fall which reached its trough usually at the 40 minute sample, and the subsequent return which was more clearly abnormal in its rapid rise, as expressed in the high values of the insulin resistance index. All curves were abnormal. The mean insulin resistance index in these twelve "untreated" acromegalics was 170, range 145-228, (mean ± 2 SD = 116-224).

3. Treated acromegalics. (Table I)

The augmented I.T.T. had returned to the normal range in eight non-diabetic treated acromegalics, five of whom were all those judged in clinical remission of the disease. In nine acromegalics with only partial clinical remission, the test was abnormal in six.

4. "Simple" obesity. (Figures 3 and 4)

The insulin resistance shown in "simple" obesity was comparable to that found in untreated acromegalics. The mean insulin resistance index was 159 and the range 119-221, (mean ± 2 SD = 89-229).

DISCUSSION

The earliest insulin tolerance tests were done on diabetes in the hope of establishing methods for defining the insulin needs of the diabetic, but since the effect of insulin in this test varies also with the level of fasting blood sugar, such tests have been supplanted for this purpose by various combined glucose and insulin

tolerance tests (Himsworth and Kerr, 1939). These latter tests have overcome the problem of interpreting the response with abnormal fasting blood sugars, and have been able to sub-divide diabetics broadly into insulin-resistant and insulin-sensitive types. However, the present augmented I.T.T. has been developed not for classifying diabetics into types, but in order to enable the demonstration of moderate or severe insulin resistance among subjects with normal fasting blood sugars. Indeed an important precaution to be observed in its use, is that comparison with the normal response is only valid when the test starts from a normal fasting blood sugar. The need for a clinical test of the metabolic abnormality of the non-diabetic acromegalics, in order to assess the effectiveness of Au-198 and Y-90 implants in this disease, has lead us to define the normal response of this augmented I.T.T. Such a test might have other useful clinical applications, especially now that the attention of diabetic research is being focussed on pre-diabetes, and on the problem of insulin antagonists (Annotation, Brit. Med. J., 1961).

It seems clear that for this purpose more insulin should be given than was used in the standard test evolved for the demonstration of panhypopituitarism. The chosen dose of thrice the standard dose, or 11.1 units of soluble insulin per square meter of body surface, was found to be free from untoward effects in any of our subjects, but should not be used in subjects with subnormal insulin resistance. If the normal range which we have defined is to be

used elsewhere, the standardised conditions of the test must be carefully followed: careful preliminary exclusion of other intercurrent disease, the dietary preparation, the exact length of the preliminary fast and nearly basal conditions throughout the test. Our results have shown the great importance of assessing the presence of obesity, for a marked abnormality may be found in gross "simple" obesity.

The major practical clinical application of the augmented I.T.T. is to assess the "activity" of untreated acromegalic cases, and their response to appropriate treatment. The usefulness of this test as an indirect measure of growth hormone activity is stressed by the absence of any other equivalent test of growth hormone activity (Joplin et al., 1961).

To express in physiological terms what the test measures is a much more complex problem. Many non-endocrine factors, including the nutritional state and the liver function, affect this response to insulin, and hence the importance of a careful preparation of the patient for the test. Only the first few minutes of the initial fall of the blood sugar curve reflect the unantagonised effect of insulin in enhancing peripheral glucose utilisation (Monaco et al., 1959). The speed of the subsequent return of the curve towards the fasting blood sugar reflects mainly the adequacy of the subject's insulin antagonists, and involves increased hepatic glucose output as much as peripheral antagonism of the action of the insulin. It is thus rather loose usage to call the abnormality

demonstrated by this test, insulin resistance, but there is no convenient alternative term. The main hormones antagonistic to the action of insulin on carbohydrate metabolism are the anterior pituitary growth hormone and cortisol or similar adrenal steroids (Houssay, 1936; de Bode and Altszuler, 1958; Fraser, 1960). The insulin sensitivity of the hypophysectomised animal depends mainly on growth hormone lack, though cortisol lack also contributes (de Bode and Altszuler, 1958). We may, therefore, anticipate that excess of growth hormone, or acromegaly, might be one of the most potent factors likely to lead to excess insulin resistance. It has indeed been shown that acromegalics show a decreased response in peripheral uptake of glucose on injection of insulin (Butterfield et al., 1961; Rabinowitz and Zierler, 1961a). The former showed that this also involved the decreased fixation of radioactive insulin in the forearm after brachial artery injection. Other metabolic abnormalities in acromegaly probably also contribute to the insulin resistance which they show.

Insulin resistance in obese subjects has previously been demonstrated (Arendt and Pattee, 1956), but it was important to assess the extent of this abnormality on the standard test. Obesity is associated with excessive hormonal activity, as has been shown by Rabinowitz and Zierler (1961b), who found evidence suggesting hyperinsulinism from measurements of the basal forearm metabolism, while others have found evidence of cortisol overproduction by steroid measurements (Cohen, 1958; Eckert, Green and Migeon, 1961).

Whether over-production of these and other hormones in obesity is sufficient to explain the excessive insulin resistance shown in this condition remains a problem.

SUMMARY

1. An augmented insulin tolerance test and its normal response is described; after a standard preparation, 11.1 units of soluble insulin per square meter of body surface is injected intravenously, and the blood sugar followed for two hours.
2. In such states as acromegaly the test can demonstrate insulin resistance by an abnormally rapid return of the blood sugar curve to normal. The curve can be summarised by an "insulin resistance index", the sum of the blood sugar values were mean 92 and range 62-142. The test is only valid if the fasting blood sugar is normal.
3. Test results are reported in (a) twelve "untreated" acromegalics in all of whom the test showed an abnormal insulin resistance index (mean 173 and range 144-228); (b) fourteen treated acromegalics (after Au-198 or Y-90 implants), in whom eight tests were normal, five being all the cases judged to be in clinical remission; (c) twelve subjects with gross but "simple" obesity in whom the test results were nearly as abnormal as those of the "untreated" acromegalics (mean insulin resistance index 159 and range 119-221).
4. The test can be used to assess the "activity" of acromegaly and its response to treatment, provided the subject is not also grossly obese.

REFERENCES

Annotation: Diabetes mellitus and hormone imbalance. Brit. Med. J. 1961, 2, 1484.

Arendt, E.C., and Pattee, C.J. Studies on obesity I. The insulin-glucose tolerance curve. J. clin. Endocrin. 1956, 16, 367.

Butterfield, W.J.H., Garratt, C.J., Rees, J.R., and Wichelow, M.J. Studies of insulin fixation *in vivo* in normal subjects, acromegalics and diabetics, and its effect on local glucose metabolism. 44th Int. Diab. Congr (Geneva) 1961, Communication 136.

Ciba Colloquia on Endocrinology. Immunoassay of Hormones. 1962, in press.

Cohen, H. 17-ketogenic steroid excretion in obese children before and after weight reduction. Brit. Med. J. 1958, 1, 686.

Csepai, K., and Ernst, Z. Beitrage zur kenntnis der beziehungen zwischen schilddruse und pankreas. Munch. Med. Wschr. 1927, 74, 1757.

de Bodo, R.C., and Altszuler, N. Insulin hypersensitivity and physiological insulin antagonists. Physiol. Rev. 1958, 38, 389.

Eckert, J.P., Green O.C., and Migeon, C.J. Rate of production and degradation of cortisol in obesity. 43rd. Annual Meeting of the Endocrine Society, June, 1961, Abstract No. 76, p. 41.

Fraser, T.R.C. M.D. Thesis "The diagnosis of Simmonds' disease" (New Zealand University), 1945.

Fraser, T.R.C. Endocrine disorders and insulin action. Brit. med. Bull. 1960, 16, 242.

Fraser, T.R.C., Albright, F., and Smith, P.H. Carbohydrate metabolism. J. clin. Endocrin. 1941, 1, 297.

Himsworth, H.P., and Kerr, R.B. Insulin and alimentary hyperglycemia in young normal subjects. Clin. Sci. 1939, 4, 1.

Houssay, B.A. Carbohydrate metabolism. New Engl. J. Med. 1936, 971.

Joplin, G.F., Fraser, R., Steiner, R., Laws, J., and Jones, E. Partial pituitary ablation by needle implantation of gold-198 seeds for acromegaly and Cushing's disease. Lancet, 1961, 2, 1277.

Monaco, R.N., Anderson, G.E., and Kaulbach, M. Insulin sensitivity in organic hyperinsulinism: diagnosis of insulinoma. Metabolism, 1959, 8, 49.

Rabinowitz, D., and Zierler, K.L. Forearm metabolism in obesity and its response to intra-arterial insulin. Evidence for adaptive hyperinsulinism. Lancet, 1961, 2, 690.

Rabinowitz, D., and Zierler, K.L. Forearm metabolism in acromegaly. Proceedings of the 44th. meeting of the Endocrine Society, Chicago, June, 1962, Abstract 63, p. 31.

Read, G.H., and Bryan, C.T. Immunological assay of human growth hormone. Recent Progr. Hormone Res. 1960, 16, 187.

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Figure 2 - The augmented I.T.T. in "untreated" acromegaly (mean and range) compared with normal subjects (mean ± S.D.).

Figure 3 - The augmented I.T.T. in "simple" obesity (mean and range) compared with "untreated" acromegaly (mean ± S.D.).

Figure 4 - Insulin resistance index from augmented I.T.T. in normal subjects, "untreated" and treated acromegaly, and "simple" obesity (mean ± 2 S.D. and individual values).

Table I.

The augmented I.T.T. in Au-198 or Y-90 treated acromegalics.

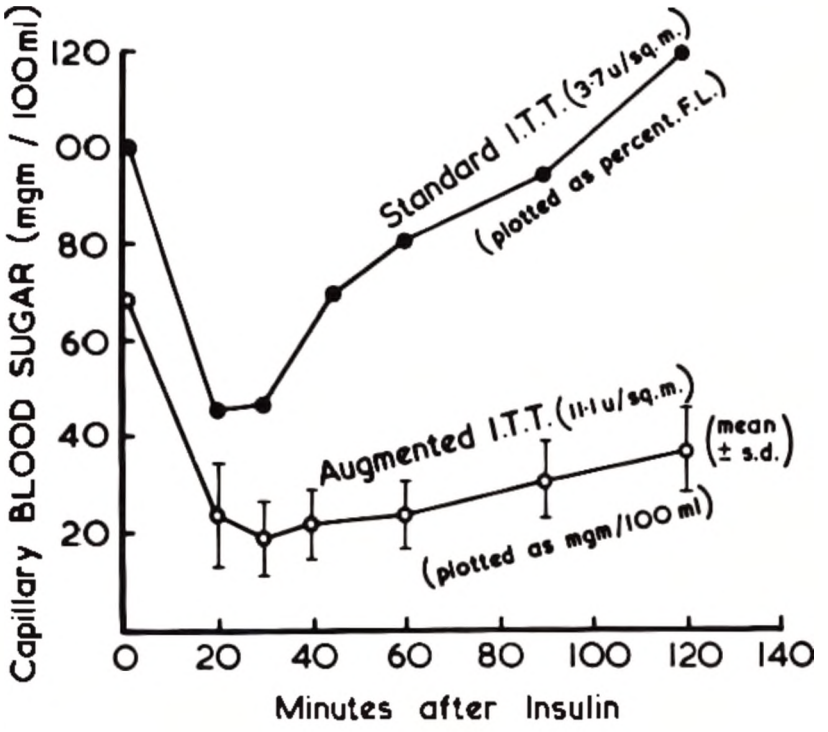
Subject	Capillary blood sugars (mgm/100 ml) time in minutes after insulin injected			Insulin Resistance Index (=A+B+C)
	60 (A)	90 (B)	120 (C)	
(a) Clinically remitted				
C.S. (3)	24	33	46	102
J.F.	33	38	46	117
S.R.	20	24	24	68
K.B.	37	41	48	126
S.C.	29	34	38	101
(b) Partially remitted				
C.S. (2)	41	60	72	173
F.E.	53	73	73	199
M.W.	37	54	76	167
H.L.	50	59	76	185
A.B.	36	59	66	161
M.S.	30	43	53	131
R.S.	45	58	64	167
M.F.	33	42	61	136

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A.B.	36	59	66	161
M.S.	30	43	53	131
R.S.	45	58	64	167
M.F.	33	42	61	136

Normal Subjects



(Dec '61)

FIG. 1.

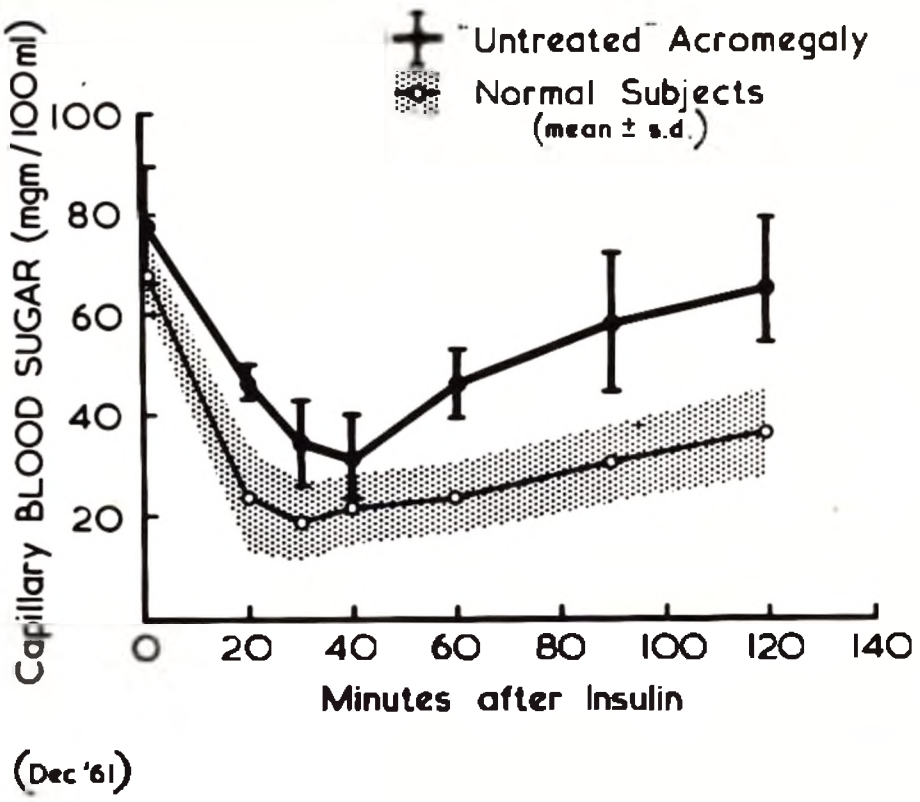

"Untreated" Acromegaly
Normal Subjects
(mean ± s.d.)
Capillary BLOOD SUGAR (mgm/100ml)
100
80
60
40
20
0
20
40
60
80
100
120
140
Minutes after Insulin
(Dec '61)

FIG. 2.

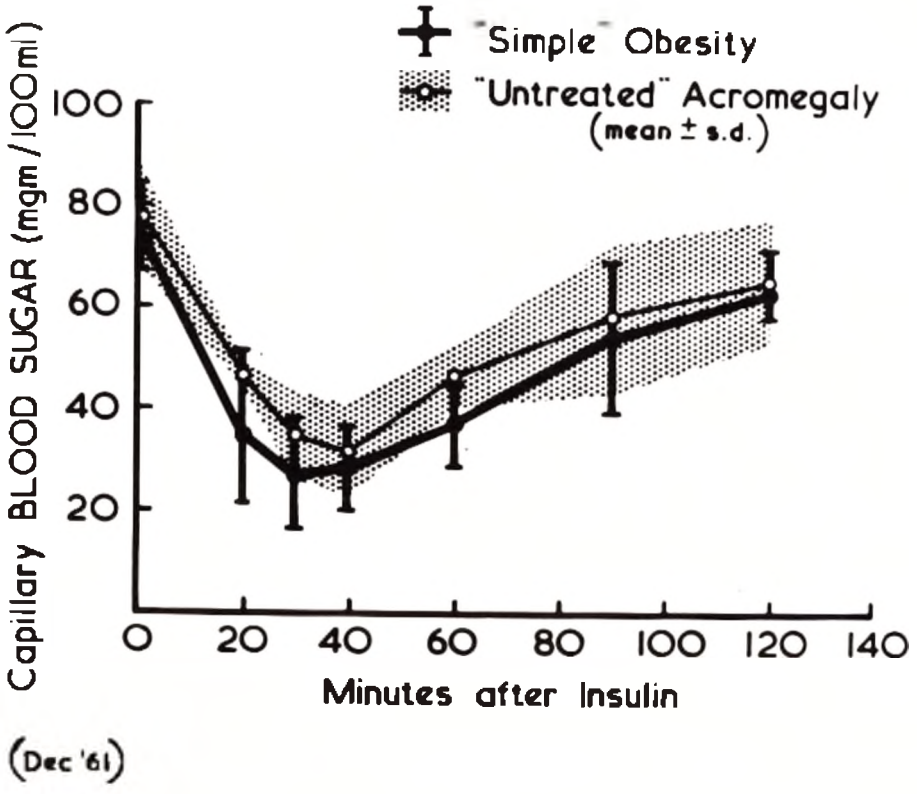
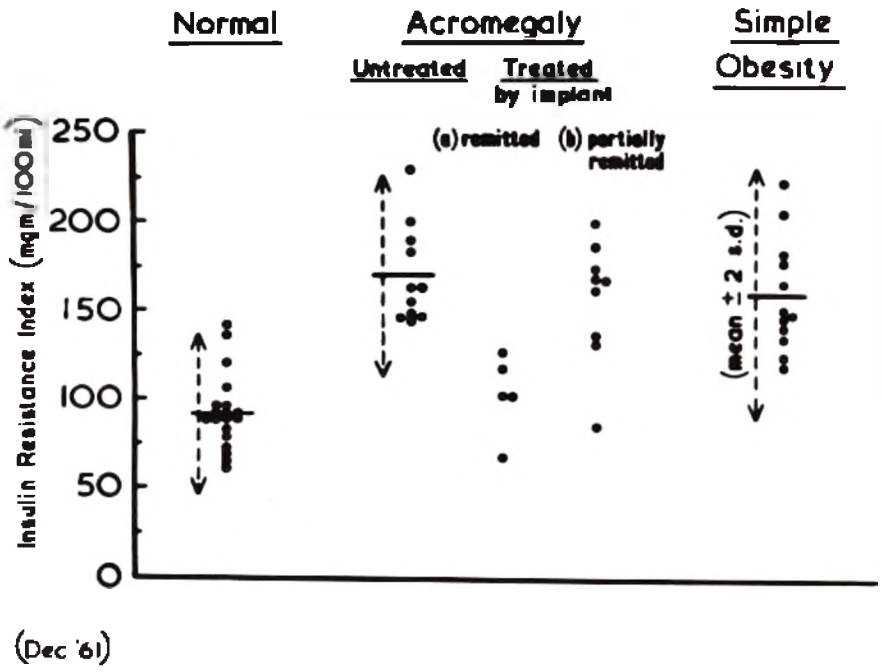

"Simple" Obesity
"Untreated" Acromegaly
(mean ± s.d.)
Capillary BLOOD SUGAR (mgm/100ml)
100
80
60
40
20
0
20
40
60
80
100
120
140
Minutes after Insulin
(Dec '61)

FIG. 3.

INSULIN RESISTANCE INDEX

(from augmented Insulin Tol. Test)



(Dec '61)

FIG. 4.

CHAPTER IV

FOREARM METABOLISM IN OBESITY AND ITS RESPONSE TO INTRA-ARTERIAL INSULIN. CHARACTERIZATION OF INSULIN RESISTANCE AND EVIDENCE FOR ADAPTIVE HYPERINSULINISM*

* Some aspects of this work have been published (1,2).

Obesity is considered ordinarily to be a consequence of overeating by an otherwise normal person, but the notion persists that there may be some underlying metabolic defect. The man of 70 kg, on becoming 20 per cent overweight, doubles his adipose organ mass (3). If obesity is viewed as a state of chronic substrate excess necessitating increase in fat storage of this magnitude, one might indeed predict that certain metabolic adjustments would occur, resulting in alterations in, but not implying a primary disturbance of, intermediary metabolism.

Metabolism of obese subjects has been examined by measuring metabolic events in the forearm, which is predominantly skeletal muscle and adipose tissue, in the search for answers to the following questions. 1) What departures from normal take place in basal forearm metabolism of obese subjects and what light do these differences shed on adaptation to chronic substrate excess? 2) Is "simple" obesity related to maturity onset diabetes mellitus, in which obesity is common? Because resistance to exogenous insulin occurs in untreated maturity onset diabetes (4), response to intra-arterial insulin was examined in simple obesity. From the observations it became clear that there are a number of similarities in metabolism between patients with simple obesity and those with diabetes mellitus.

METHODS

Six young subjects were studied, four men and two women, all approximately 20 per cent overweight but otherwise healthy. All

had been actively gaining weight in the 6 to 12 months preceding the test and in none was there a family history of diabetes. Results of oral glucose tolerance tests with 100 g glucose, performed in five of the six subjects, were normal. Subjects were given a 200-g carbohydrate diet for the 3 days before the test. No food was permitted after 8:00 p.m. the previous night. The test was performed between 9:00 a.m. and 1:00 p.m., that is, 13 to 17 hours after the last meal.

A brachial artery, an ipsilateral antecubital vein draining mostly muscle, and a superficial vein draining mostly forearm adipose tissue and skin were cannulated by techniques described previously (5). Blood flow was measured by the indicator-dilution method, based on continuous injection of T-1824 (Evans' blue) dye at constant rate. Circulation to the wrist and hand was excluded during experimental periods by a sphygmomanometer cuff inflated to at least 100 mm Hg beyond systolic pressure.

Uptake or production of a metabolite by the forearm is given by the equation $\dot{Q} = F(A - DV)$, where F is blood or plasma flow in milliliters per minute per 100 ml of forearm, A is concentration of metabolite in arterial blood or plasma, and DV is its concentration in deep venous blood. This $\dot{Q}$ will be referred to as "forearm muscle metabolic rate" even though it underestimates true muscle metabolism. To obtain $\dot{Q}$ per 100 g of muscle, it is necessary to know the fraction of total flow that perfuses muscle and the fraction of total forearm that is muscle mass. With representative figures of 0.82 and 0.6, respectively, for these fractions

in normal subjects (5), $\dot{Q}$ per 100 g of muscle is obtained by multiplying the experimental value of $\dot{Q}$ (shown later in Tables I and II) by a factor, f, of 1.37. The greater proportion of adipose tissue in the forearm of subjects who are 20 per cent overweight may reduce the fraction of total forearm volume occupied by muscle to about 0.5. The fraction of total flow that perfuses muscle in the obese forearm is probably less than 0.82, but probably now as low as 0.67, for this is the figure at which f in obese subjects is equal to that for controls. For values of forearm muscle flow between 0.67 and 0.82 of total forearm flow, f, varies between 1.37 and 1.6 in subjects who are 20 per cent overweight; that is, f is greater in obese subjects than in controls. Therefore, wherever experimental values for forearm metabolism are greater in obese subjects than in controls (for example, $\dot{Q}$ glucose, Table I), the differences from controls ought to be revised upward.

At least two sets of arterial, deep venous, and superficial venous blood samples were collected under basal conditions, and all values for basal metabolism have been expressed as their means. To shed further light on previous observations (6) that obese subjects show resistance to the hypoglycemic effects of intravenously administered insulin, we have examined the response of the forearm of these 6 obese subjects to a dose of intra-arterial insulin, standardized previously in normal subjects (7). Glucagon-free insulin was injected, along with Evans' blue dye, into the brachial artery in a dose of 100 μU per kg body weight per minute for 26 minutes. When this dose is diluted by brachial arterial plasma

flowing by the injection site, the concentration of insulin in forearm arterial plasma is approximately 300 μU per ml, the exact concentration depending upon forearm blood flow during the experimental period. Circulation to the wrist and hand was occluded during the full period of insulin administration. Blood samples were drawn 12, 18, and 26 minutes from commencement of the insulin infusion, and for periods up to an hour thereafter.

The technique lends itself nicely to examination of local effects of hormones, since the hormone--insulin in the present experiments--can be injected into the ipsilateral brachial artery at constant rate in amounts large enough to give measurable effects on the forearm, but small enough to give none in the general circulation. Thus, one observes as far as possible only the effects of the hormone on forearm tissues; the response is not, as in the case of the more conventional intravenous insulin tolerance tests, complicated by counter-regulatory measures the body may institute.

RESULTS AND DISCUSSION

Basal forearm muscle metabolism.

Results appear in Tables I and II. Control values, which represent mean values of more than 60 experiments, are listed for comparison (8).

Arterial concentrations.

There were no significant differences in the mean arterial concentrations of the metabolites studied in the obese group compared to the control values. The mean arterial concentrations of

FFA was 0.79 mEq per L in the obese subjects; while there have been reports of increased venous FFA levels in obese subjects (9), we have not, in a small series, demonstrated consistent arterial hyperlipacidemia.

Blood flow.

Total forearm blood flow in obese subjects did not differ from control subjects. We are unable to estimate what the fractional flow is through muscle and through subcutaneous adipose tissue and skin.

Potassium uptake.

Net movement of potassium out of resting muscle into plasma, characteristic in control subjects under the conditions of these experiments (8, 10), was absent in the obese subjects, who had no net movement of potassium.

Glucose uptake.

Mean glucose A - DV concentration difference and mean glucose uptake by forearm muscle were both significantly greater than those of controls.

O_2 uptake, CO_2 production, and respiratory quotient (R.Q.)

No differences from controls were apparent in O_2 consumption or CO_2 production by forearm muscle in obese subjects. The RQ of 0.7, as in normal muscle, is compatible with lipid oxidation.

A - DV FFA difference.

Mean FFA A - DV concentration difference in obese subjects was + 0.02 μEq per ml, which does not differ from the mean value in control subjects (8). Direct demonstration that this fraction

of plasma lipid serves as a major substrate for forearm muscle in obese subjects has not been possible. The anatomical factors believed to underly this have been discussed in detail elsewhere (8). In brief, it is probable that release into deep venous blood of FFA from adipose tissue surrounding muscle masks the positive A - V FFA concentration difference across forearm muscle proper. Since there is undoubtedly a greater mass of adipose tissue in the obese forearm, it is of some interest that A - DV FFA differences were not more negative in the obese subject than in controls.

The probable metabolic fate of glucose.

The L/G ratio--which, as explained in a footnote to Table II, defines the fraction of glucose uptake accounted for by lactate production--was significantly lower in forearm muscle of obese subjects (0.24) than in controls (0.42). Seventy-five per cent of glucose abstracted from blood, therefore, remains unaccounted for and theoretically available for complete oxidation. This yields a G - L/O_2 ratio (see footnote to Table II)--which defines the fraction of oxygen uptake theoretically accountable for by glucose oxidation of 0.34, or approximately twice that of controls.

Since, however, forearm muscle in obese subjects takes up more glucose than controls without any rise in RQ, it is probable that the glucose taken up in excess of that abstracted by controls is not oxidized but stored, presumably largely as muscle glycogen.

Forearm skin and subcutaneous tissue metabolism.

Mean values for basal arterio-superficial venous (A - SV) concentration differences of glucose, lactate, potassium, and FFA

in the six obese subjects appear in Table III, along with control values (8). Although mean A - SV concentration differences for potassium, glucose, and lactate were changed in a direction similar to changes in A - DV, t tests of these changes suggested ($p > 0.05$) that they occurred by chance. Mean A - SV difference for FFA was, however, significantly less negative than in controls.

Evidence for endogenous hyperinsulinism.

In control subjects, A - DV concentration differences reflect chiefly metabolism of muscle and A - SV, chiefly metabolism of skin and subcutaneous adipose tissue. A - SV glucose differences are greater (more positive) and A - SV O_2 differences are smaller (less positive) than the respective A - DV differences. Lactate and FFA SV - A differences are greater (more negative), whereas potassium SV - A differences are smaller (less negative) than the respective DV - A differences (8).

It is conceivable but unlikely that differences between control and obese subjects with respect to A - DV concentrations and values for $\dot{Q}$ represent nothing more than the increased contribution to DV effluent of blood draining the larger mass of adipose tissue in the obese forearm. If this effect were responsible for the increase in $\dot{Q}$ of glucose and absence of negative $\dot{Q}$ of potassium in obese subjects, then A - DV differences of all metabolites in obesity ought to show the same tendency to resemble A - SV differences of controls. Our studies do not, however, support this interpretation: A - DV O_2, CO_2, and lactate differences in obese subjects did not differ from

A - DV concentrations of control subjects, but clearly differed from their A - SV concentrations (Tables I and II).

These findings also negate the possibility that our observations merely represent redistribution of blood between deep and superficial forearm drainage beds. This would uniformly obscure or eliminate the differences normally found in A - V concentration differences of metabolites from the two drainage beds. A - DV O_2, CO_2, and FFA differences clearly differ from A - SV differences in obese subjects. (Compare Tables I and II with Table III.)

It is probable, therefore, that deviations from normal forearm metabolism in obese subjects can not be attributed solely either to alterations in forearm blood flow or to the undoubtedly greater mass of adipose tissue in the obese forearm.

Previous studies from this laboratory (7) have demonstrated that insulin infused into the brachial artery into control subjects reverses the net potassium efflux and increases glucose uptake by forearm muscle. Only a minor fraction of the glucose is accounted for by lactate production and none is oxidized to CO_2. The RQ of forearm muscle does not rise. The effect of insulin on the superficial drainage bed of the forearm is to increase A - SV glucose concentration difference, with a fall in the L/G ratio, and to convert basal negative A - SV FFA differences to a net positive A - SV difference (11). Metabolic events in the forearm of obese subjects in the absence of exogenous insulin resemble those in the normal forearm exposed to insulin, and suggest that in obesity there is hyperinsulinism that is presumably adaptive to unusual quantities

of substrate. This hypothesis was advanced by Brobeck, Tepperman, and Long (12) some years ago and gained support from the demonstration of islet cell hyperplasia in the early or "active" phase of obesity by Ogilvie in man (13) and by Mayer (14) in rats.

We are grateful to Drs. S.A. Berson and R.S. Yalow for determining for us by their immunoassay technique (15) plasma insulin concentrations in our six obese subjects and in four normal subjects of the same age group. Results appear in Table IV. Plasma insulin concentration was higher in obese subjects than in those of normal body weight ($p < 0.01$), although insulin concentration in our normal group may be slightly less than in the over-all experience of Yalow and Berson (15).

Effect of insulin on forearm muscle metabolism.

Results appear in Table V.

Insulin concentration in forearm plasma.

This is established by determining the ratio of the known rate of injection of insulin, 100 μU per kg per minute, to forearm plasma flow measured by Evans' blue dye. Values varied from 200 to 450 μU per ml, levels comparable to those achieved in an earlier study on control subjects (7).

Plasma flow.

It has been pointed out repeatedly that both blood flow and arterial concentration must remain constant for proper employment of the Fick principle (16). Plasma flow before and during the period of insulin infusion remained acceptably constant; that is,

it did not vary by more than 20 per cent about the mean in any of the subjects studied. In one subject, P, a marked increase in plasma flow occurred in the period after the insulin infusion.

Arterial concentrations.

Mean changes of less than 4 per cent were recorded in arterial glucose, lactate, and potassium concentrations during and after insulin administration. Arterial concentration of FFA tended to be more unstable than other metabolites, as is consistent with the much greater lability of this plasma lipid fraction.

Glucose.

Glucose uptake in obese subjects after insulin administration was distinctly less than in controls (Figure 1). In only one of the six obese subjects did the A - DV concentration difference exceed 1 µmole per ml, and the maximal uptake achieved was always less than 5 µmoles per minute per 100 ml forearm, which was the lowest peak effect recorded in any of the control subjects (7).

Lactate.

There was a small but definite increase in lactate production during and after insulin administration. Since glucose uptake increased to a greater extent than lactate production, the L/G ratio dropped from a control value of 0.24 to less than 0.10.

$\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, and RQ.

Observations on O_2 consumption, CO_2 production, and RQ were fragmentary only, being confined to the 26 minute sample, taken just before the conclusion of the insulin infusion, and the 60

minute sample. Such measurements as were made showed no change in either $\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, or RQ which corresponds with our experience in control subjects.

Potassium.

In the basal state, the net movement of potassium out of forearm muscle into venous blood is absent in obese subjects. Administration of insulin resulted in mean potassium A - DV differences becoming more positive. Total movement of potassium achieved under the influence of exogenous insulin was significantly less in obese subjects than the comparable change in control subjects (Figure 2).

FFA.

A - DV concentration difference of FFA increased from a mean basal value not significantly different from 0 to 0.1 μEq per ml after insulin administration. This effect is probably achieved through inhibition of FFA release from adipose tissue interspersed between muscle fibres. Uptake of FFA by forearm muscle unmasked by insulin administration is sufficient to account for about 50 per cent of its O_2 consumption.

Effect of insulin on forearm skin and subcutaneous adipose tissue metabolism.

Values for A - SV concentration differences of glucose, lactate, and FFA after insulin administration, determined in 5 obese subjects, are shown in Table VI. In the sixth subject, L, the SV catheter was accidentally dislodged.

Glucose.

Glucose A - SV differences increased after insulin administration, but significantly less so than in controls.

Lactate.

There was a small but significant increase in lactate SV - A differences. The L/G ratio dropped from 0.31 to 0.22.

FFA.

Insulin effectively inhibited release of FFA from forearm adipose tissue in obese subjects. Insulin-induced change in A - SV FFA concentration difference was significantly less in obese subjects than in controls (Figure 3).

The apparent paradox of endogenous hyperinsulinism and insulin resistance in obesity.

In early obesity there is increased circulating insulin in the basal state and the obese subjects, judged by the observations of forearm metabolism, do respond to this increased endogenous insulin. Yet they resist, i.e., respond less than normals to administered insulin. This apparent paradox can be resolved only if it can be shown either that they are not really resistant to exogenous insulin or that they are in fact also resistant to their own insulin.

For the first solution, we have considered only the possibility that they might appear superficially to resist exogenous insulin if the quantity administered were superimposed on such a large pool that the relative increase in concentration is small

compared to that after administration of the same amount to normal subjects. The obese subject, moving up the dose-response curve only slightly, would then display only a small metabolic response to insulin, appropriate to the normal dose-response curve, and would not be truly insulin resistant. This hypothesis, however, is untenable in that the quantity of insulin administered to obese subjects was sufficient to raise forearm arterial insulin concentration by about 15 times, so that if they were not truly insulin-resistant, as we have defined the phrase, they ought to have responded quantitatively like normal subjects.

The second solution to the paradox is more likely, i.e., obese subjects may be resistant to their own insulin. We have no data on the insulin dose-response curve describing forearm metabolism, so we can not say that normal subjects, in whom insulin concentration was as high as that found endogenously in obese subjects, would respond to a greater extent, but we suspect that this is the case. This suspicion is not weakened by the report by Karam, Grodsky, and Forsham (17) that in obesity an apparently normal response to glucose tolerance tests occurs only at the expense of an abnormally large increase in circulating insulin. This is reminiscent of the pattern described by Yalow and Berson (15) in maturity onset stable diabetes.

Relationship between simple obesity and maturity onset stable diabetes.

About 80 per cent of patients with maturity onset stable diabetes are obese (18). It is apparent that some of the features associated with this condition are shared by subjects with simple obesity, for

example, elevation of plasma insulin concentration (15), resistance to exogenous insulin with respect to glucose movement into forearm muscle (19) and a greatly exaggerated plasma insulin response to a glucose load (17). The following sequence is suggested: chronic substrate excess in obesity causes adaptive hyperinsulinism, leading ultimately to "high output failure" of the pancreatic islets. In line with this, Ogilvie (20)found that whereas glucose tolerance was usually normal in the early phases of obesity, it was abnormal in all subjects in whom obesity had been present for longer than 18 years. In John's series (21), evidence of glucose intolerance was present in over half the adult obese subjects he examined, but this was not the case in obese children. The factors that determine whether the obese subject adapts successfully or not to chronic substrate excess remain to be established. One important link is probably provided by the recent demonstration by Vallance-Owen and Lilley (22) of an antagonist to insulin in the plasma of obese prediabetics.

Possible factors producing insulin resistance in simple obesity.

There remains the question of why insulin resistance occurs in obesity. Eckert, Green, and Migeon (23) found cortisol production increased in simple obesity. Although this suggests that insulin resistance in obesity may result from cortisol antagonism, it is unlikely that this is the case. In disturbances of the hypophyseal-adrenocortical axis, including Cushing's syndrome (24), the metabolic pattern of insulin resistance differs from that in obesity. In

obesity, there is resistance to all the metabolic effects of insulin we have studied, but in disease of the hypophyseal-adrenocortical axis, expressions of insulin resistance are limited to less responsiveness with respect to glucose and potassium uptake by the forearm tissues, and insulin-induced inhibition of FFA release from forearm adipose tissue is at least normal.

We are left with no really completely satisfying explanation of insulin resistance in obesity. It is possible that resistance in this case is simply a manifestation of tolerance to chronic (endogenous) administration of insulin.

Whatever the explanation, insulin resistance appears to be a consequence of obesity and not a primary defect. It was reversed after shedding of excess adipose tissue in one subject (unpublished observations by Andres, Baltzan, and Zierler) and Newburgh and Conn (24) found that, even in later stages of obesity, abnormal responses to glucose tolerance tests disappeared after weight loss.

SUMMARY

1. Forearm metabolism (glucose and potassium uptake and lactate production by muscle, and release of free fatty acids from adipose tissue) in obese subjects not receiving insulin resembles that of normal subjects in whom insulin is infused intra-arterially. From this effect, confirmed by assay of plasma insulin concentration, it is likely that hyperinsulinism accompanies early obesity.

2. The respiratory quotient of forearm muscle in obese subjects is 0.7, as in normal muscle, which is compatible with oxidation of lipid.

3. Intra-arterial administration of insulin produces significantly less glucose and potassium uptake by forearm muscle and significantly less glucose uptake and movement of free fatty acids in forearm adipose tissue in obese subjects than in controls.

4. These studies may provide a link in the chain of evidence incriminating simple obesity as a precursor of maturity onset stable diabetes.

REFERENCES

1. Rabinowitz, D., and Zierler, K.L. Forearm metabolism in obesity and its response to intra-arterial insulin. Physiologist 1961, 3, 89.
2. Rabinowitz, D., and Zierler, K.L. Forearm metabolism in obesity and its response to intra-arterial insulin. Evidence for adaptive hyperinsulinism. Lancet 1961, 2, 690.
3. Keys, A., Brozek, J. Body fat in adult man. Physiol. Rev. 1953, 33, 245.
4. Himsworth, H.P. The mechanism of diabetes mellitus. III. Human diabetes mellitus. Lancet 1939, 2, 171.
5. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.
6. Arendt, E.C., and Pattee, C.J. Studies on obesity I. The insulin-glucose tolerance curve. J. clin. Endocr. 1956, 16, 367.
7. Andres, R., Baltzsan, M.A., Cader, G., and Zierler, K.L. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J. clin. Invest. 1962, 41, 108.
8. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.
9. Dole, V.P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. clin. Invest. 1956, 35, 150.

10. Andres, R., Cader, G., Goldman, P., and Zierler, K.L. Net potassium movement between resting muscle and plasma in man in the basal state and during the night. J. clin. Invest. 1957, 36, 723.

11. Rabinowitz, D., and Zierler, K.L. Effect of insulin on free fatty acid metabolism in the human forearm. Fed. Proc. 1962, 21, 200.

12.8 Brobeck, J.R., Tepperman, J., and Long, C.N.H. Experimental hypothalamic hyperphagia in the albino rat. Yale J. Biol. Med. 1943, 15, 831.

13. Ogilvie, R.F. The islands of Langerhans in 19 cases of obesity. J. Path. Bact. 1933, 37, 473.

14. Mayer, J. The obesity hyperglycemic syndrome of mice as an example of "metabolic" obesity. Amer. J. clin. Nutr. 1960, 8, 712.

15. Yalow, R.S., and Berson, S.A. Immunoassay of endogenous plasma insulin in man. J. clin. Invest. 1960, 39, 1157.

16. Zierler, K.L. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. J. clin. Invest. 1961, 40, 2111.

17. Karam, J.H., Grodsky, G.M., and Forsham, P.H. Excessive insulin response to glucose in obese subjects as measured by immunochemical assay (abstract). Proc. Amer. Diab. Ass., June, 1962. p. 12.

18. Joslin, E.P., Root, H.F., White, P., Marble, A., and Bailey, C.C.

The treatment of diabetes mellitus, 8th edition. Philadelphia, Lea and Febiger, 1946, p. 70.

19. Andres, R. and Zierler, K.L. Spontaneous and insulin-induced resistance of peripheral tissues to insulin in diabetes. Clin. Res. 1958, 6, 250.

20. Ogilvie, R.F. Sugar tolerance in obese subjects. A review of sixty-five cases. Quart. J. Med. 1935, 4, 345.

21. John, H.J. Glucose tolerance studies in children and in adolescence. Endocrinology, 1934, 18, 75.

22. Vallance-Owen, J. and Lilley, M.D. Insulin antagonism in the plasma of obese diabetics and prediabetics. Lancet 1961, 1, 806.

23. Eckert, J.P., Green, O.C., and Migeon, C.J. The rate of production and degradation of cortisol in obesity (abstract). Proc. 43rd meeting Endocr. Soc. June, 1961, p. 41.

24. Rabinowitz, D., and Zierler, K.L. Dissociation of insulin action in the forearm of man (abstract). J. clin. Invest. 1962, 41, 1393.

25. Newburgh, L.H. and Conn, J.W. A new interpretation of hyperglycemia in obese middle-aged persons. J. Amer. Med. Ass. 1939, 112, 7.

Table I

Forearm Metabolism in Obesity

Subject	Glucose			Potassium			FFA	
	A	A-DV	$\dot{Q}$	A	A-DV	$\dot{Q}$	A	A-DV
Z	4.92	0.11	0.61	3.92	+ 0.06	+ 0.19	0.60	+ 0.08
G	5.31	0.35	1.08	3.85	+ 0.04	+ 0.07	0.58	+ 0.02
L	5.47	0.18	0.44	3.83	+ 0.03	+ 0.04	1.26	0.00
W	4.56	0.22	0.91	3.82	+ 0.05	+ 0.12	0.90	- 0.06
P	5.31	0.06	0.57	4.10	- 0.05	- 0.20	0.74	- 0.01
F	5.25	0.34	0.72	4.17	- 0.08	- 0.09	0.67	+ 0.07
Mean	5.10	0.21	0.72	3.95	+ 0.01	+ 0.02	0.79	+ 0.02
±S.E.M.	0.142	0.048	0.132	0.048	0.007	0.058	0.107	0.021
Controls*	4.96	0.14	0.52	4.02	- 0.19	- 0.43	0.71	+ 0.02
±S.E.M.	0.051	0.017	0.075	0.026	0.027	0.076	0.052	0.034
P value	N.S.	< 0.05	< 0.05	N.S.	< 0.001	< 0.001	N.S.	N.S.

Values for arterial concentrations and A-DV differences are expressed as μM/ml. Uptake and production data ($\dot{Q}$) are expressed as μM/min/100 ml forearm. P is probability that difference between mean in control and in obese group might occur by chance, estimated by *t* test. N.S., meaning not significant, is applied to $P > 0.05$.

*See reference 8.

Table II

Blood Flow and Metabolism of Forearm Muscle in Obese Subjects

	Controls	Obese Subjects	P*
Blood Flow l/min/100 ml arm	3.7 ± 0.17	4.3 ± 1.03	N.S.
Lactate, A-DV μM/ml	- 0.12 ± 0.012	- 0.10 ± 0.014	N.S.
$\dot{Q}_{Lactate}$ μM/min/100 ml arm	- 0.44 ± 0.060	- 0.35 ± 0.074	N.S.
Oxygen, A-DV μM/ml	3.02 ± 0.168	2.88 ± 0.336	N.S.
$\dot{Q}_{O_2}$ μM/min/100 ml arm	11.6 ± 0.97	11.0 ± 1.43	N.S.
CO_2, A-DV μM/ml	- 2.4 ± 0.20	- 2.0 ± 0.245	N.S.
$\dot{Q}_{CO_2}$ μM/min/100 ml arm	- 8.7 ± 0.94	- 6.9 ± 1.03	N.S.
R.Q.	0.76 ± 0.020	0.70 ± 0.045	N.S.
L/G** per cent	42.3 ± 8.3	24.3 ± 6.7	< 0.05
G-L/O_2*** per cent	16.1 ± 4.6	33.3 ± 7.5	< 0.05

All values are means ±S.E. of means.

*See legend to Table I.

**See Table II (continued)

***See Table II (continued)

Table II (continued)

Blood Flow and Metabolism of Forearm Muscle in Obese Subjects

** L/G = 100 (1/2 lactate arteriovenous difference/glucose arteriovenous difference). Since production of 2 moles of lactate implies dissimilation of 1 mole of glucose, this ratio yields the percentage of glucose uptake accounted for by lactate production. Mean value for L/G is calculated from the mean lactate A-DV difference and the mean glucose A-DV difference and its S.E.M. is calculated from variance of A-V lactate and of A-V glucose differences.

*** $G\text{-}L/O_2$ = 100 (glucose arteriovenous difference - 1/2 lactate arteriovenous difference)/1/6 oxygen arteriovenous difference. Since that fraction of glucose, unaccounted for by lactate production, is available for complete oxidation and since 6 moles of oxygen are required for complete combustion of 1 mole of glucose, this ratio yields the fraction of oxygen accounted for if all the glucose abstracted from blood were completely combusted. Mean value for $G\text{-}L/O_2$ is calculated from the mean glucose A-V difference, mean lactate A-V difference and mean O_2 A-V difference.

Table III

A-SV Differences in Obese Subjects and in Controls

	Controls	Obese Subjects	P*
Potassium	- 0.03 ± 0.021 (33)	+ 0.01 ± 0.026	N.S.
Glucose	+ 0.19 ± 0.027 (26)	+ 0.23 ± 0.047	N.S.
Lactate	- 0.18 ± 0.015 (33)	- 0.14 ± 0.020	N.S.
L/G**	47.3 ± 7.6 (33)	31.6 ± 8.3	< 0.05
Oxygen	1.93 ± 0.273 (5)	1.55 ± 0.24	N.S.
CO_2	- 1.56 ± 0.285 (5)	- 1.45 ± 0.178	N.S.
FFA	- 0.13 ± 0.028 (34)	- 0.07 ± 0.020	< 0.05

Data, which are expressed as μM/ml, are means ±S.E. of means. Number of control subjects shown within parentheses. Control values for O_2 and CO_2 A-SV differences are means ±S.E. of means of a previously unreported study of 5 young subjects of normal body weight.

*See legend to Table I.

**See legend to Table II.

Table IV

Arterial Plasma Insulin Concentrations

in Control and Obese Subjects

	Controls		Obese
	5	W	15.5
	9	Z	19.0
	10.5	G	36.0
	11.5	F	29.0
		P	46.0
		L	42.0
Mean	9.0		31.3
S.E. of means	1.43		4.96

$P < 0.01$

Plasma insulin concentrations were determined by Drs. S. A. Berson and R. S. Yalow by their immuno-assay technique (15). All values which are the means of two independent assays are expressed in μU/ml. P is probability that difference between mean in control and in obese group might occur by chance, estimated by t test. Controls were 4 normal non-obese young men from whom blood samples were obtained in the basal state 15 hours after the last meal.

Table V

Mean Effect of Insulin on Forearm Metabolism in Obesity

Time after insulin started min	Plasma Flow ml/min/100 ml arm		A-DV FFA µM/ml		A-DV Lactate µM/ml	
	Obese	Control	Obese	Control	Obese	Control
0	2.6±0.62	2.1±0.14	+0.02±0.021	+0.02±0.038	-0.10±0.015	-0.14±0.026
12	2.5±0.42	2.7±0.32	+0.04±0.024	+0.02±0.022	-0.12±0.071	-0.16±0.035
18	2.9±0.84	2.6±0.26	+0.05±0.027	+0.06±0.021	-0.08±0.022	-0.16±0.026
26	2.7±0.55	3.1±0.28	+0.09±0.020	+0.08±0.014	-0.12±0.024	-0.22±0.034
40	2.6±0.10	2.7±0.24	+0.09±0.020	+0.09±0.033	-0.13±0.038	-0.24±0.042
60	3.9±1.70	2.6±0.32	+0.09±0.020	+0.02±0.041	-0.15±0.027	-0.24±0.034

Insulin administered intra-arterially for 26 minutes beginning at time zero. Values for obese group (6 subjects) are means ±S.E. of means. Control data on plasma flow and lactate A-DV differences are means ±S.E. of means among 10 young subjects of normal body weight (7). Control values for FFA A-DV differences (and for all control data reported in Table VI) are means ±S.E. of means of a previously unreported study of 5 young subjects of normal body weight. Reported control plasma flows cannot therefore be employed to calculate $\dot{Q}_{FFA}$ for control subjects.

Table VI

Mean Effect of Insulin on A-SV Concentrations of Glucose, Lactate and FFA in Obesity

Time after insulin started min	A-SV Glucose μM/ml		A-SV Lactate μM/ml		A-SV FFA μM/ml	
	Obese	Control	Obese	Control	Obese	Control
0	0.23±0.047	0.17±0.013	-0.14±0.020	-0.15±0.015	-0.07±0.020	-0.16±0.033
12	0.21±0.044	0.43±0.048	-0.17±0.009	-0.12±0.011	-0.06±0.010	+0.02±0.054
18	0.27±0.054	0.65±0.060	-0.13±0.014	-0.17±0.029	+0.01±0.036	+0.06±0.016
26	0.36±0.078	0.83±0.038	-0.17±0.031	-0.23±0.030	+0.02±0.032	+0.06±0.020
40	0.30±0.084	0.80±0.032	-0.13±0.044	-0.21±0.035	+0.02±0.014	+0.09±0.023
60	0.35±0.039	0.50±0.044	-0.16±0.012	-0.24±0.037	+0.03±0.020	-0.03±0.013

Insulin administered intra-arterially for 26 minutes beginning at time zero. Values for obese group (5 subjects) are means ±S.E. of means. Control values are means ±S.E. of means of a previously unreported study of 5 young subjects of normal body weight.

Fig. 1 Effect of insulin on forearm glucose uptake in obese subjects. Shaded area represents mean ± S.E. of mean among 10 control subjects (7). Individual lines indicate glucose uptake in obese subjects identified by initials.

Fig. 2 Effect of insulin on forearm potassium uptake in obese subjects, expressed as the increase in net inward movement of potassium compared to potassium movement at time zero. Shaded area represents mean ± S.E. of mean among 10 control subjects (7). Individual lines indicate change in potassium movement in obese subjects identified by initials.

Fig. 3 Effect of insulin on FFA A - SV concentration differences in obese subjects, expressed as the change in FFA A - SV concentration difference compared to A - SV difference at time zero. Shaded area represents mean ± S.E. of mean among 5 previously unreported control subjects of normal body weight. Individual lines indicate change in A - SV differences in obese subjects identified by initials.

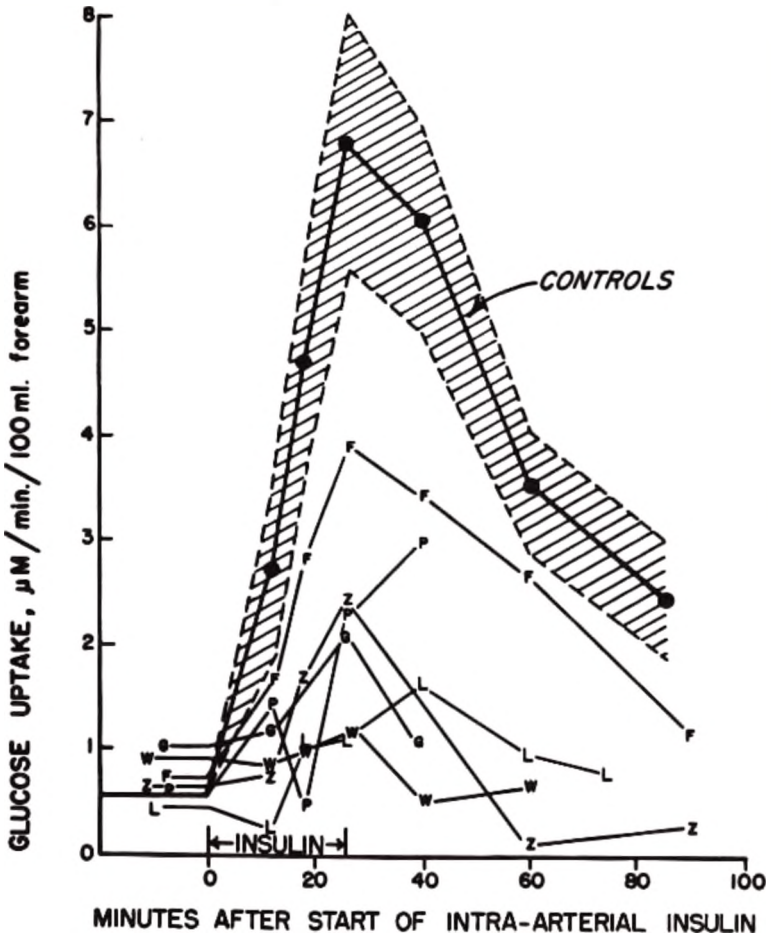

GLUCOSE UPTAKE, μM/min./100ml. forearm
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN
CONTROLS
INSULIN
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100

FIG. 1.

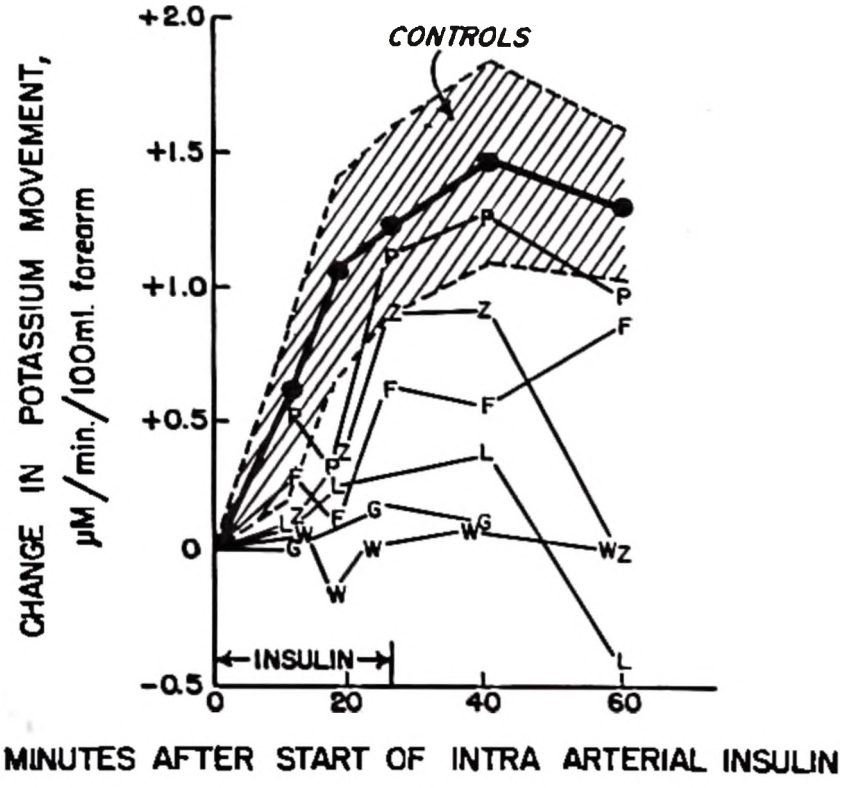

CONTROLS
+2.0
+1.5
+1.0
+0.5
0
-0.5
INSULIN
0
20
40
60
CHANGE IN POTASSIUM MOVEMENT, μM/min./100ml. forearm
MINUTES AFTER START OF INTRA ARTERIAL INSULIN

FIG. 2.

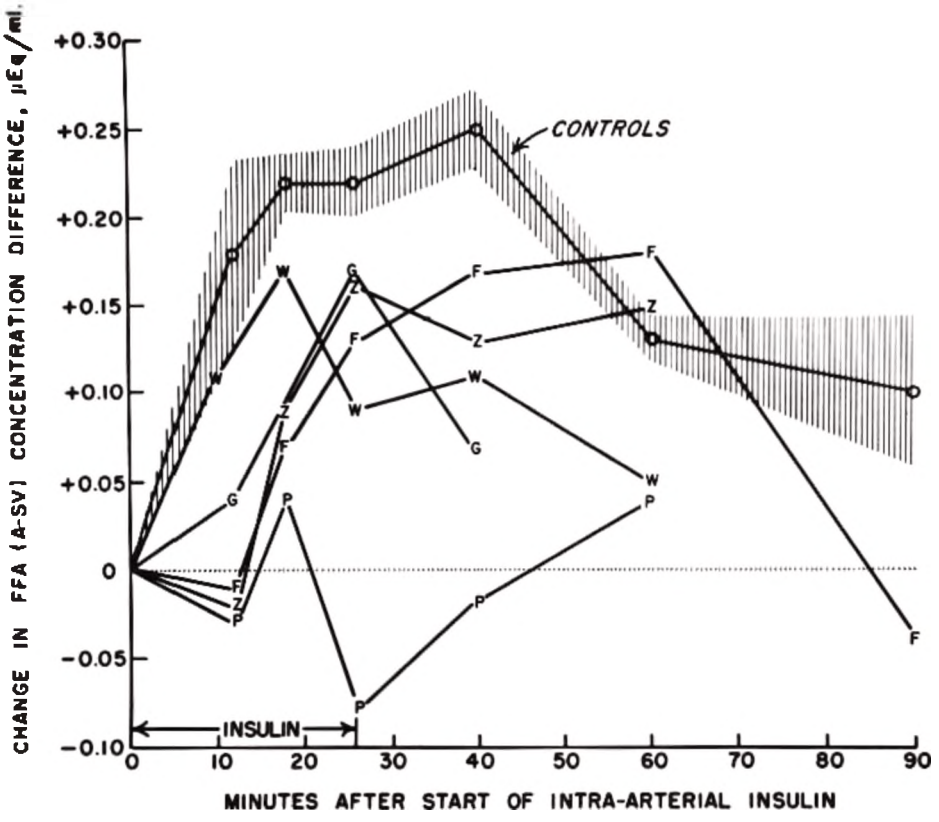

CHANGE IN FFA (A-SV) CONCENTRATION DIFFERENCE, μEq/ml.
+0.30
+0.25
+0.20
+0.15
+0.10
+0.05
0
-0.05
-0.10
CONTROLS
INSULIN
0
10
20
30
40
50
60
70
80
90
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 3

CHAPTER V

FOREARM METABOLISM IN ACROMEGALY BEFORE AND AFTER THERAPY

The gross clinical features of acromegaly and their association with pituitary basophilic adenomata have been well recognised for many years (1, 2). Hypersecretion of growth hormone (HGH), and perhaps other related hormones by basophilic tumors of the hypophysis, does not appear to continue indefinitely; after a variable period of time, a proportion of acromegalic tumors tend to become inactive (3). In most patients, however, obvious acromegalic features persist. Recent advent of a variety of sound therapeutic measures for dealing with the tumor (4) has increased the importance of establishing objectively whether or not the tumor is active; that is, whether it continues to hypersecrete HGH. In the absence of either extrasellar extension provoking hemianopsia or frank enlargement of acral parts, a clinical decision as to activity is extremely difficult. Furthermore, the laboratory methods hitherto available for indirectly testing for excessive circulating HGH have in general proved inadequate. Neither the level of serum phosphorus nor the B.M.R. as conventionally measured, has consistently correlated with activity. Methods to gauge the anti-insulin properties of HGH by observing venous glucose concentrations after administration of a standard dose of insulin intravenously, have proved difficult to interpret, and often do not provide a definitive answer (5, 6), since insulin resistance with respect to glucose is shared by a wide number of different states, including obesity.

In the light of this experience, the pattern of forearm metabolism in acromegaly was investigated. Answers were sought to the following questions: (1) What departures from normal are found in basal forearm metabolism of acromegalic subjects prior to therapy, and how can this be related to increased circulating HGH in these subjects: (2) What is the response of the forearm of acromegalic subjects to close intra-arterial injection of insulin? (3) What changes occur in basal forearm metabolism and response to insulin following therapy?

METHODS

Six acromegalic subjects were investigated eight times. All subjects were studied between the hours of 8 A.M. and noon, 12 to 16 hours after their last meal. History of carbohydrate intake was normal. The brachial artery, and a draining deep and superficial forearm vein were catheterised by techniques previously described (7). Forearm blood flow was measured by indicator-dilution techniques based on continuous infusion of Evans blue dye T-1824 into the brachial artery. Arterio-deep venous (A - DV) concentration differences reflect the metabolism of forearm muscle in the main. Uptake or production of a metabolite by forearm tissues, chiefly muscle, is given by the equation $\dot{Q} = F(A - DV)$ where $\dot{Q}$ = uptake (or production) of the metabolite in units of mass per minute per 100 ml forearm, F = blood (or plasma) flow in units of ml per minute and A and DV are concentrations of the metabolite in arterial and deep venous blood (or plasma) respectively.

A - SV concentration differences reflect the metabolism of forearm skin and subcutaneous adipose tissue chiefly.

At least two sets of control samples were taken. Insulin was then infused into the ipsilateral brachial artery in a concentration previously standardised in control subjects, viz. 100 μunits per kg per minute for 26 minutes (8). The concentration achieved in brachial arterial plasma, usually between 300 and 700 μunits per ml, is large enough to affect local forearm metabolism without, however, provoking any systemic response. Samples were taken 12, 18 and 26 minutes following the start of the infusion, and for periods up to one hour thereafter.

RESULTS AND DISCUSSION

Forearm metabolism in active acromegaly:

Results appear in Tables I and II.

Blood flow: Although mean forearm blood flow in acromegalic subjects was about 25 per cent higher than the mean value among control subjects (9), t-test of this difference was not significant ($P > 0.05$).

Glucose uptake: Mean glucose uptake by forearm muscle ($\dot{Q}_G$) in acromegaly did not differ quantitatively from that of controls (9).

Potassium uptake: Net movement of potassium out of resting muscle into plasma, characteristic in control subjects under the conditions of our experiments (9), was absent in acromegalic

subjects, who all showed net uptake of potassium by forearm muscle. Positive potassium balance has been repeatedly reported following administration of HGH in man (10, 11), and net inward movement of potassium into forearm muscle has been observed following direct intra-arterial infusion of HGH (12).

Oxygen uptake: Mean oxygen consumption ($\dot{Q}_{O_2}$) by forearm muscle in acromegalic subjects was about 50 per cent above mean control values. Because oxygen consumption by forearm muscle is extremely brisk in control subjects, with an extraction ratio of 0.4 or greater (9), an increase of this magnitude in acromegaly becomes very meaningful. Elevation of B.M.R. as determined by conventional methods, has been frequently reported in acromegaly (13); direct determination of oxygen consumption by peripheral forearm tissues, principally muscle, supports this observation.

Respiratory Quotient: The respiratory quotient of about 0.7, as in normal muscle, is compatible with lipid oxidation.

FFA metabolism: Acromegalic subjects exhibited large negative A - DV and A - SV FFA concentration differences. This probably reflects respectively an enhanced rate of FFA release from subcutaneous adipose tissue and from the adipose tissue intimately interspersed among forearm muscle bundles. These observations are consistent with a number of independent investigations reporting convincing hyperlipacidemia in man

and animals (14, 15) following the administration of HGH by either I.M. or I.V. injection. Contrasting with these in vivo effects, has been the demonstration that growth hormone possesses poor or erratic lipolytic activity when exposed to excised rat adipose tissue under appropriate in vitro conditions (16). This suggests that HGH may not be directly responsible for mobilisation of FFA from adipose tissue.

Response of forearm to intra-arterial insulin.

The response of the forearm in acromegaly to close intra-arterial injection of insulin has been described in detail elsewhere (17, 18). In brief (a) glucose uptake by forearm muscle is reduced to less than half normal (Fig. 1); (b) potassium movement into muscle is similarly reduced (Fig. 2); (c) insulin-induced glucose and potassium uptake by forearm adipose tissue is reduced, but there is probably less inhibition than there is of uptake by muscle; and (d) insulin-induced inhibition of FFA release from forearm adipose tissue is at least normal (Fig. 3).

Resistance to insulin-induced uptake of glucose by forearm has been previously described in acromegaly by Butterfield's group (19), and by Ginsberg, Galbraith and Paton (20). However, in acromegaly, as in other situations characterised by hypophyseal-adrenocortical axis disturbance, a distinctive dissociation of insulin action is seen, in which the effect of insulin in checking release of fatty acids from adipose tissue is retained. This contrasts with the global resistance to insulin exhibited by simple obese subjects (21).

Acromegaly in remission.

Two subjects have been restudied following therapy. Subject B was restudied 10 months after interstitial implantation of 35 mc. radio-gold (Au^{198}) into the pituitary. Subject S was re-studied 4 months after surgical hypophysectomy. No evidence of pituitary hypofunction was present in either.

Results and Discussion.

Basal forearm metabolism: Basal forearm metabolism of subjects S and B, both before and after therapy, appear in Table III.

Potassium uptake: The characteristic net movement of potassium out of muscle, absent prior to therapy, was restored post-operatively.

Oxygen uptake: Oxygen consumption by forearm muscle, elevated prior to therapy, was restored to normal in both subjects.

FFA movement: The large negative A-V FFA concentration differences noted prior to therapy were reduced (i.e., became less negative) toward normal in both subjects.

Response of the forearm to intra-arterial insulin:

Glucose and potassium uptake: Glucose uptake by forearm muscle in response to intra-arterial insulin administration showed a significant increase over the pre-therapy response in both subjects (Figs. 4 and 5). Increase in net movement inward of potassium after insulin administration was similarly significantly increased compared with the pre-therapy values (Figs. 6 and 7).

FFA movement: In active acromegaly insulin-induced FFA movement is normal. As anticipated, insulin effect on FFA movement was unchanged following therapy.

DISCUSSION

The departures from normal in basal forearm metabolism of acromegalic subjects reflect, in part, the consequence of chronic growth hormone over-production on the tissues of man. Increase in O_2 consumption by forearm, net potassium uptake by forearm muscle, and enhanced release of FFA from forearm adipose tissue are compatible with the observations made after administration of milligram quantities of HGH to man (10, 11). Similar effects have been observed following local intra-arterial injection of HGH into the human forearm (12). HGH administration also produces a prompt decrease in both glucose uptake and lactate production by forearm tissues, neither of which is observed in acromegaly. Of course, the increased secretion of endogenous HGH in acromegaly clearly provokes counter-regulatory responses in the endocrine system. For example, HGH administration increases insulin secretion from the pancreas (22), and elevation of plasma insulin-like activity (I.L.A.) has been frequently observed in active acromegaly (23). This may well be responsible for maintaining adequate translocation of glucose from plasma into peripheral tissues in acromegaly.

Taken in concert, these deviations in forearm metabolism provide a reliable method for objectively determining "activity"

in acromegaly. All three abnormalities in forearm metabolism (elevation of O_2 consumption, net movement of K^+ into forearm muscle, and enhanced release of FFA from forearm adipose tissue) are not consistently present in each case, but two of these features are always present. Of course, with normal "physiological variation" in basal metabolism, the isolated finding of one of these features is not uncommon in control subjects.

Perhaps the single most reliable index of activity in acromegaly is evidence for dissociation of insulin action in the forearm:

Insulin-induced glucose uptake by forearm tissues is reduced to less than half normal in acromegaly, which is in agreement with similar observations by Butterfield et al (19) and Ginsberg et al (20). In none of the active acromegalics to whom insulin was given did the A - DV glucose concentration exceed 1 μM/ml, which is the lowest value seen in any of the control subjects. On the other hand, the effect of insulin on FFA movement in the forearm is normally retained in acromegaly. This dissociation of insulin action, is common to a number of other disorders of the hypophyseal-adrenocortical axis (17, 18), (Cushing's syndrome and starvation), but differs from the global resistance to insulin seen in simple obesity.

In the two patients who were restudied following adequate therapy, basal metabolism had reverted toward normal; that is,

net K^+ efflux from forearm muscle was present, and $\dot{Q}_{O_2}$ of forearm muscle and negative A - SV FFA differences were reduced. In addition, the response of forearm tissues to intra-arterial insulin with respect to glucose and potassium was largely restored to normal. Since this index is reproducable and easily quantifiable, it is likely to provide the most satisfactory single objective method for gauging the adequacy of therapy.

SUMMARY

Deviations in forearm metabolism in acromegaly (elevation of forearm O_2 consumption, net inward K^+ movement, and enhanced release of FFA from adipose tissue) and its response to intra-arterial insulin (decreased glucose and potassium movement into muscle, but retention of insulin effect on FFA movement) serve as a useful method for diagnosis of activity in acromegaly.

These abnormalities revert toward normal following adequate therapy.

REFERENCES

1. Marie, P. Reprinted in Major, R.H. Classic Descriptions of Diseases, 2nd ed., Charles C. Thomas, Springfield, Ill., 1939.
2. Davidoff, L.M. Acromegaly. The anamnesis and symptomatology of one hundred cases, Endocrinology, 10, 453, 1926.
3. Mc Cullagh, E.P., and Brandon, J.M. in Clinical Endocrinology, E.B. Astwood, Ed., Grune and Stratton, Inc. 1960, p. 36.
4. Fraser, R., and Joplin, G.F. in Modern Trends in Endocrinology, H. Gardiner-Hill, Ed. Paul B. Hoeber, Inc. 1961, p. 69.
5. Fraser, R., Albright, F., and Smith, P.H. The value of the glucose tolerance test, the insulin tolerance test, and the glucose insulin tolerance in the diagnosis of endocrinologic disorders of glucose metabolism. J. clin. Endocrinol. 1941, 1, 297.
6. Fraser, R., Joplin, G.F., Opie, L.H., and Rabinowitz, D. The augmented insulin tolerance test for detecting insulin resistance. J. Endocrinol. in press.
7. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.
8. Andres, R., Baltzan, M.A., Cader, G., and Zierler, K.L. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J. clin. Invest. 1962, 41, 108.

9. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.

10. Henneman, P.H., Forbes, A.P., Moldawer, M., Dempsey, E.F., and Carroll, E.L. Effects of human growth hormone in man. J. clin. Invest. 1960, 39, 1223.

11. Clinical endocrinology committee of the medical research council. The effectiveness in man of human growth hormone. Lancet, 1959, i, 7.

12. Rabinowitz, D., and Zierler, K.L. The effect of human growth hormone on forearm metabolism in man. Unpublished observations.

13. Cushing, H., and Davidoff, L.M. Studies in acromegaly. IV The Basal Metabolism. Arch. Int. Med. 1927, 39, 673.

14. Raben, M.S., and Hollenberg, C.H. Effect of growth hormone on plasma fatty acids. J. clin. Invest. 1959, 38, 484.

15. Pearson, O.H., Dominguez, J.M., Greenberg, E.J., Pazianos, A., and Ray, B.S. Diabetogenic and hypoglycemic effects of human growth hormone. Tr. A. Am. Physicians, 1960, 73, 217.

16. White, J.E., and Engel, F.L. Lipolytic action of corticotropin on rat adipose tissue in vitro. J. clin. Invest. 1958, 37, 1556.

17. Rabinowitz, D. and Zierler, K.L. Dissociation of insulin action in the forearm of man. J. clin. Invest. 1962, 41, 1393.

18. Rabinowitz, D., and Zierler, K.L. Preservation of insulin effect on FFA movement in hypophyseal-adrenocortical dependent insulin resistance. The Physiologist, August, 1962.

19. Hardwick, C., Butterfield, W.J.H., Fry, I.K., Briggs, J.H. Direct measurement of the effect of insulin on the uptake of glucose by peripheral muscles in normal subjects, diabetics and acromegalics. Proc. Roy. Soc. Med. 1959, 52. 807.

20. Ginsberg, J., Galbraith, H.J.B., and Paton, A. Some peripheral effects of insulin and other hormones in man. The Mechanism of Action of Insulin. Edited by Broom, W.A., and Wolff, F.W. p. 225, Blackwell, 1960.

21. Rabinowitz, D., and Zierler, K.L. Forearm metabolism in obesity and its response to intra-arterial insulin. Evidence for adaptive hyperinsulinism. The Lancet, 1961, 2, 690.

22. Randle, P.J. in Diabetes, Williams, R.H. Ed. Paul B. Hoeber, Inc. 1960, p. 257.

23. Randle, P.J. Plasma insulin activity in acromegaly. The Lancet, 1954, 1, 441.

Table I

A-DV Differences in Forearm Blood and Uptake by Forearm Muscle ($\dot{Q}$) of O_2 and Potassium; Arterial Concentrations, A-DV and A-SV Differences of FFA in Acromegalic Subjects

Subject	Oxygen		Potassium		FFA		
	A-DV	$\dot{Q}$	A-DV	$\dot{Q}$	A	A-DV	A-SV
B	4.75	19.59	+0.12	+0.25	0.78		-0.44
M			+0.16	+0.72	0.55		-0.22
C	2.36	13.5	+0.08	+0.32	0.77	-0.13	-0.45
A	3.46	11.94	+0.14	+0.27	0.73	-0.11	-0.32
S	4.29	26.45	+0.12	+0.42	0.91	-0.33	-0.28
U	4.52	14.79	+0.19	+0.30	0.87	-0.04	-0.39
Mean	3.88	17.25	+0.14	+0.38	0.77	-0.15	-0.35
±S.E.M.	0.428	2.637	0.015	0.072	0.046	0.064	0.034
Controls	3.02	11.6	-0.19	-0.43	0.71	+0.02	-0.13
±S.E.M.	0.168	0.97	0.027	0.076	0.052	0.034	0.028
P value	<0.05	<0.02	<0.001	<0.001	N.S.	<0.01	<0.001

Values for A-DV concentration differences are expressed as μM/ml. Forearm uptake ($\dot{Q}$) is expressed as μM/min/100 ml arm. P is probability that difference between mean in control and in acromegalic group might occur by chance, estimated by *t* test. N.S., meaning not significant, is applied to $P > 0.05$.

Table II

Blood Flow and Metabolism of Forearm Muscle in Acromegalic Subjects

	Controls	Acromegaly	p*
Blood flow**	3.7 ±0.17	4.6 ±0.62	N.S.
$\dot{Q}$ glucose***	0.52±0.075	0.69±0.090	N.S.
$\dot{Q}$ lactate***	- 0.44±0.060	- 0.46±0.12	N.S.
$\dot{Q}$ CO_2***	- 8.7 ±0.94	-11.05±1.78	0.1>P>0.05
R.Q.	0.76±0.020	0.68±0.024	N.S.
L/G	42.3 ±8.3	39.3 ±9.4	N.S.
G-L/O_2	33.3 ±7.5	15.6 ±8.7	N.S.

All values are means ±S.E. of means. L/G = per cent of glucose uptake accounted for by lactate production. G-L/O_2 = per cent of oxygen consumption accounted for by glucose uptake corrected for lactate production.

*See legend to Table I

**Units: ml/min/100 ml arm

***Units: μM/min/100 ml arm

Table III

Forearm Metabolism in Acromegaly; Before and After Therapy

	Subject B		Subject S	
	Pre-therapy (May, 1960)	Post-therapy (March, 1961)	Pre-therapy (Dec., 1961)	Post-therapy (March, 1962)
$\dot{Q}$ glucose*	0.73	0.85	1.08	0.58
$\dot{Q}$ lactate*	0.89	1.01	0.60	0.49
$\dot{Q}$ potassium*	+ 0.25	- 0.13	+ 0.42	- 0.27
$\dot{Q}$ oxygen*	19.59	10.45	26.45	6.93
FFA (A-DV) differences**		+ 0.15	- 0.33	+ 0.14
FFA (A-SV) differences**	- 0.44	- 0.18	- 0.28	- 0.10

Values for $\dot{Q}$ lactate are negative, indicating net production by forearm tissues.

*Units: μM/min/100 ml arm

**Units: μM/ml

LEGENDS TO FIGURES

Fig. 1. Effect of insulin on glucose uptake by the forearm in active acromegaly. Each of the six subjects is identified by an initial.

Fig. 2. Effect of insulin on net inward movement of potassium in the forearm in active acromegaly. Each of the six subjects is identified by an initial.

Fig. 3. Effect of insulin on A - SV FFA differences in acromegaly. Each of the six subjects is identified by an initial.

Fig. 4. Effect of insulin on glucose uptake by forearm muscle in an acromegalic subject (T.B.) before and after therapy.

Fig. 5. Effect of insulin on glucose A - DV differences in an acromegalic subject (MLS) before and after therapy.

Fig. 6. Effect of insulin on potassium uptake by forearm muscle in an acromegalic subject (T.B.) before and after therapy. Note the differences in net basal potassium flux.

Fig. 7. Effect of insulin on potassium uptake by forearm muscle in an acromegalic subject (MLS) before and after therapy. Note the differences in net basal potassium flux.

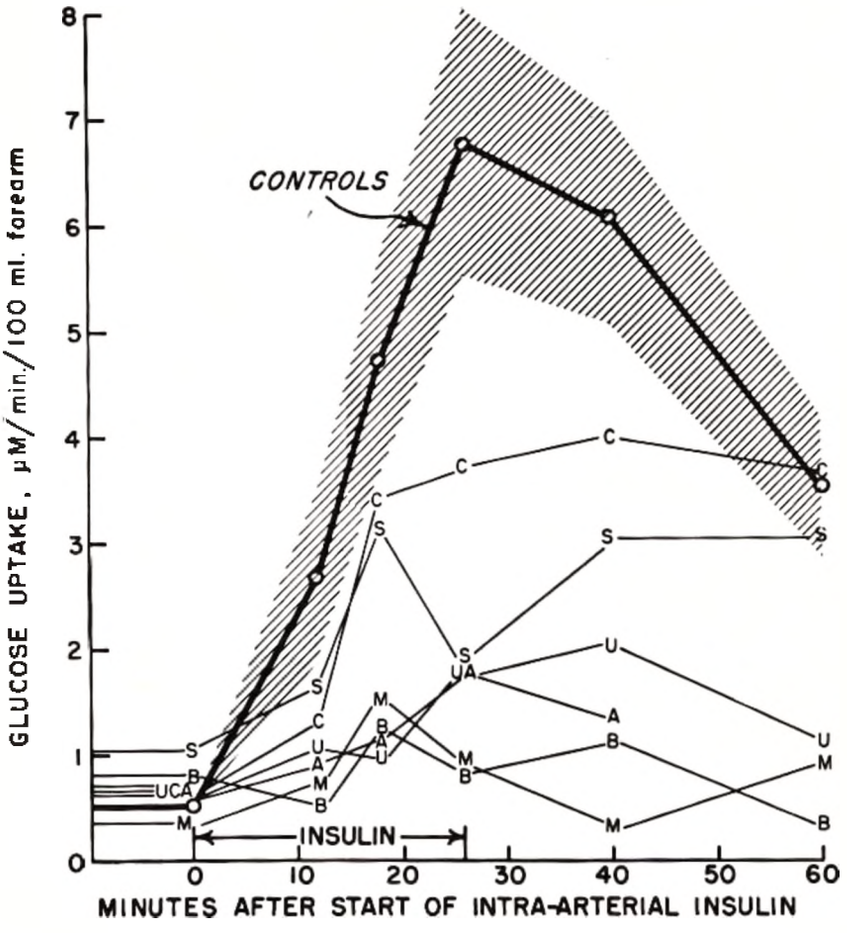

GLUCOSE UPTAKE, μM/min./100 ml. forearm
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN
CONTROLS
INSULIN
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60

FIG. 1

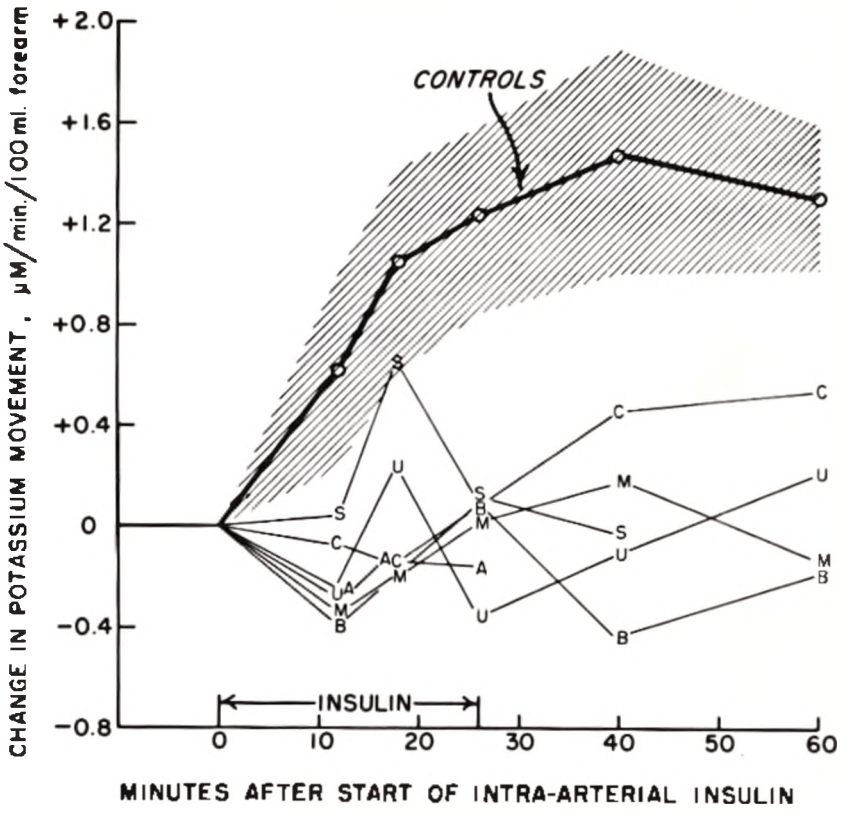

CHANGE IN POTASSIUM MOVEMENT, μM/min./100 ml. forearm
+2.0
+1.6
+1.2
+0.8
+0.4
0
−0.4
−0.8
CONTROLS
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 2.

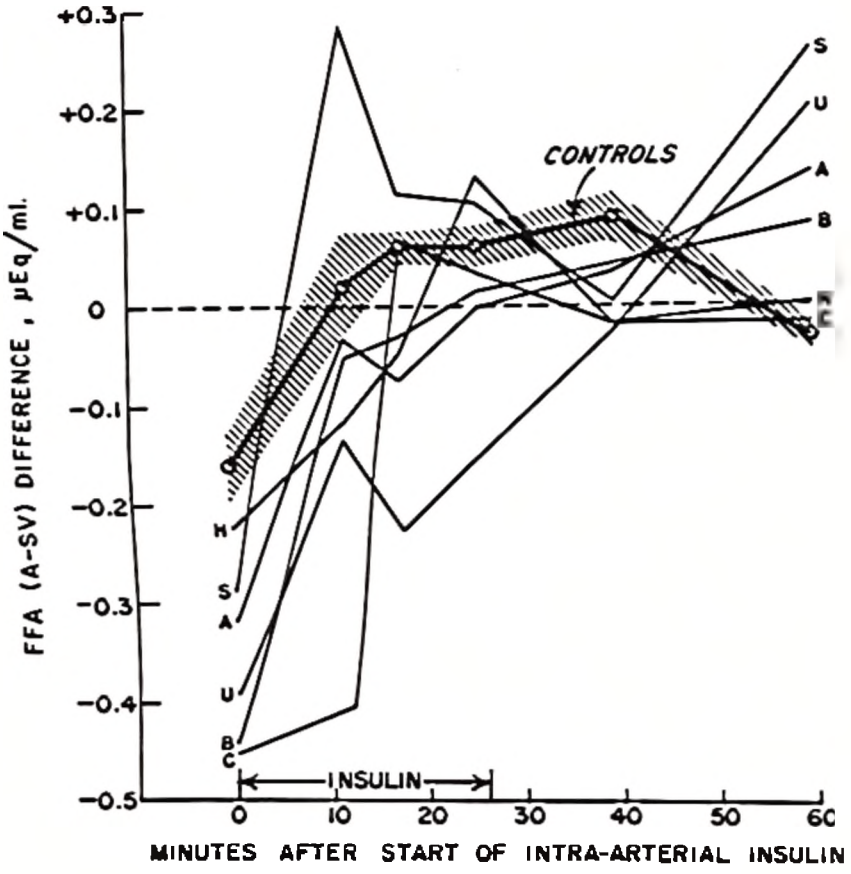

+0.3
+0.2
+0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
FFA (A-SV) DIFFERENCE , μEq/ml.
CONTROLS
S
U
A
B
H
S
A
U
B
C
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 3.

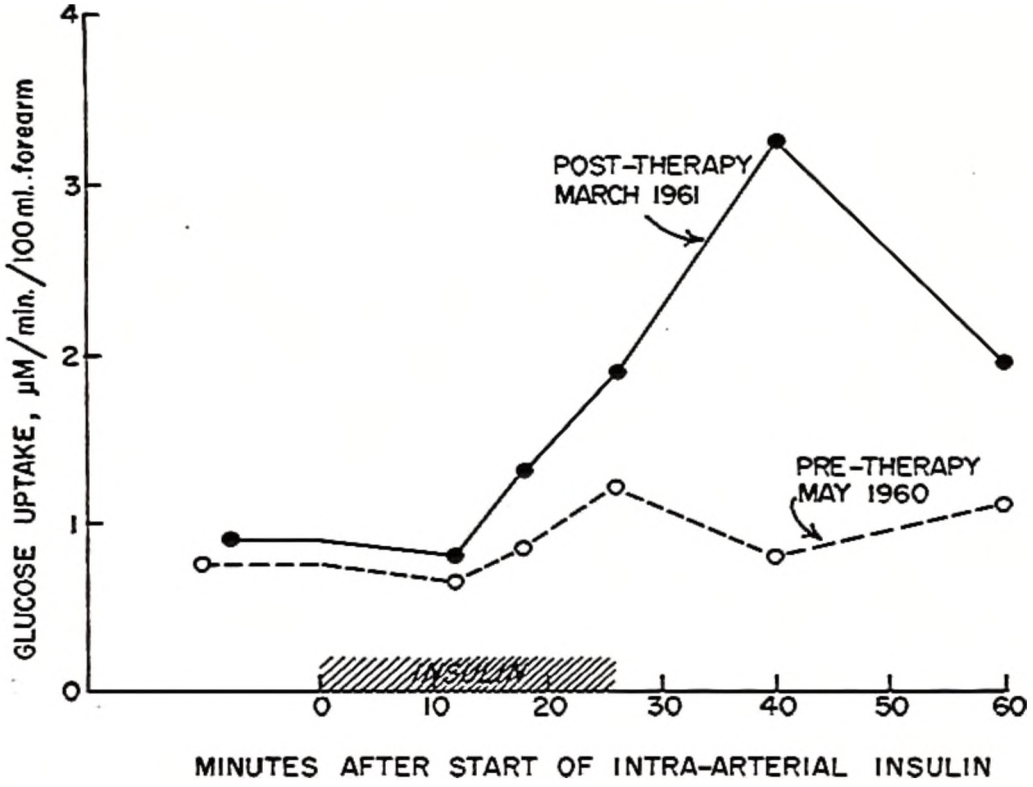

GLUCOSE UPTAKE, µM/min./100ml. forearm
4
3
2
1
0
POST-THERAPY
MARCH 1961
PRE-THERAPY
MAY 1960
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 4.

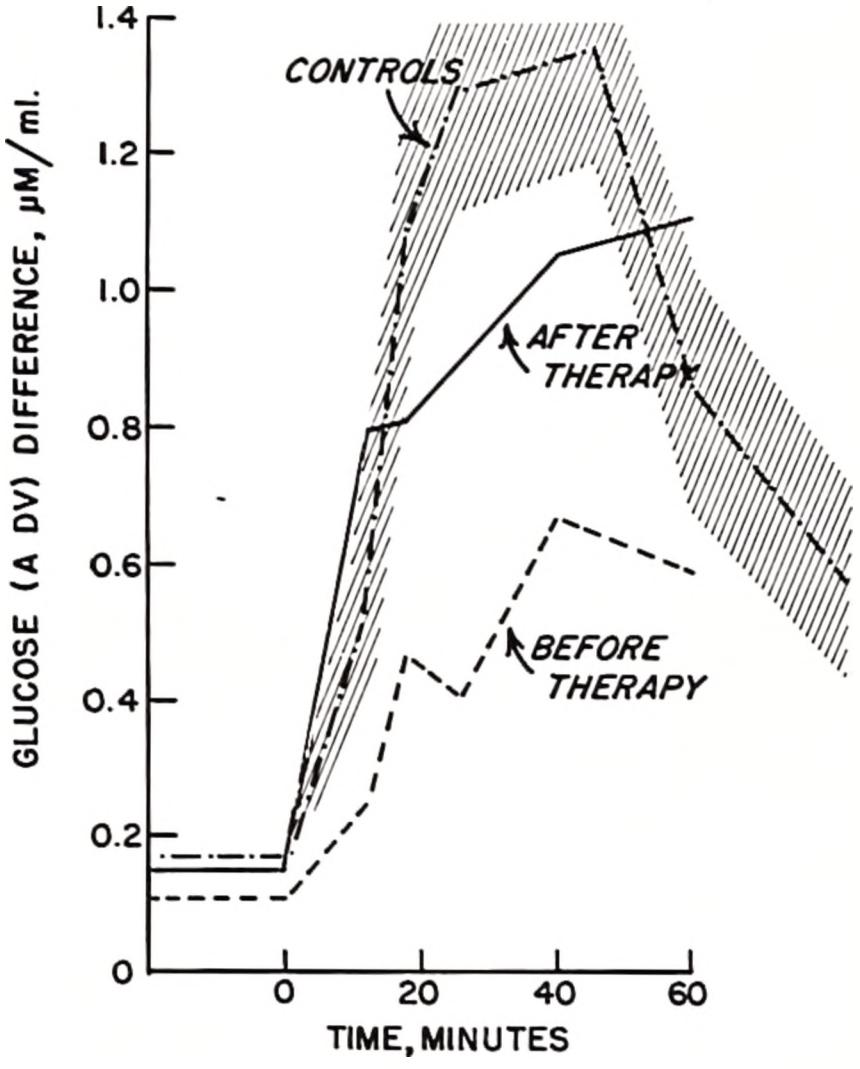

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
GLUCOSE (A DV) DIFFERENCE, μM/ml.
CONTROLS
AFTER THERAPY
BEFORE THERAPY
0
20
40
60
TIME, MINUTES

FIG. 5.

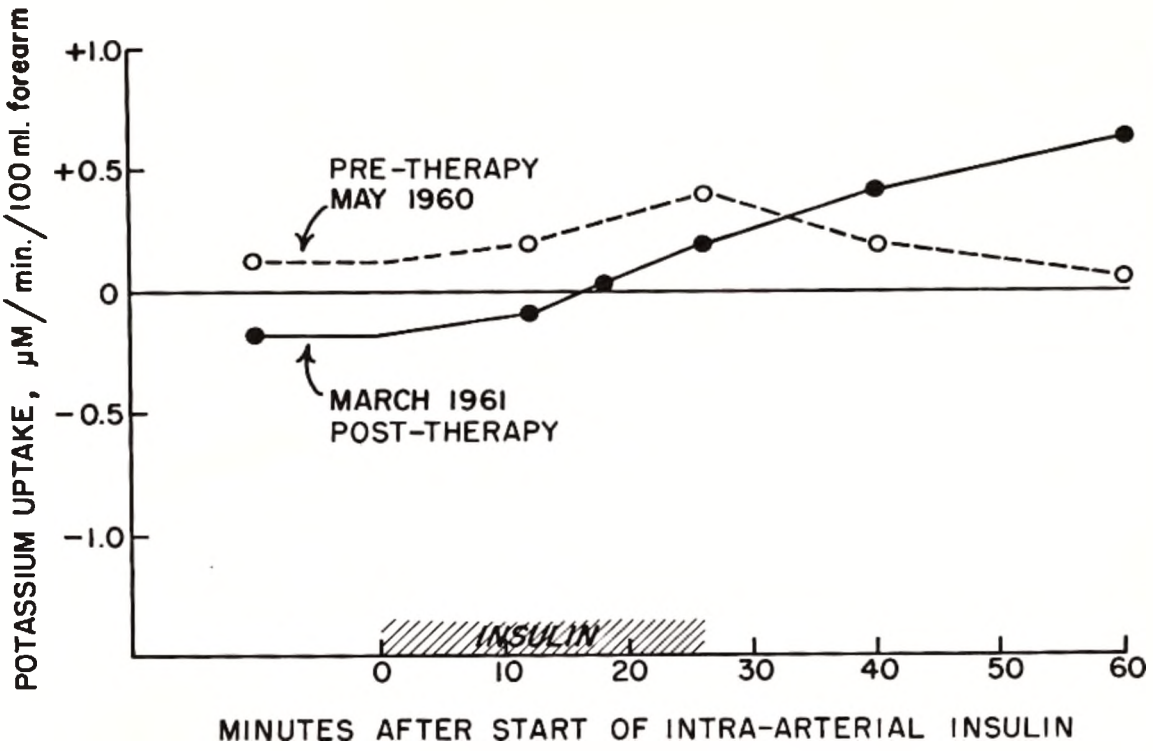

POTASSIUM UPTAKE, μM/min./100 ml. forearm
+1.0
+0.5
0
-0.5
-1.0
PRE-THERAPY
MAY 1960
MARCH 1961
POST-THERAPY
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 6.

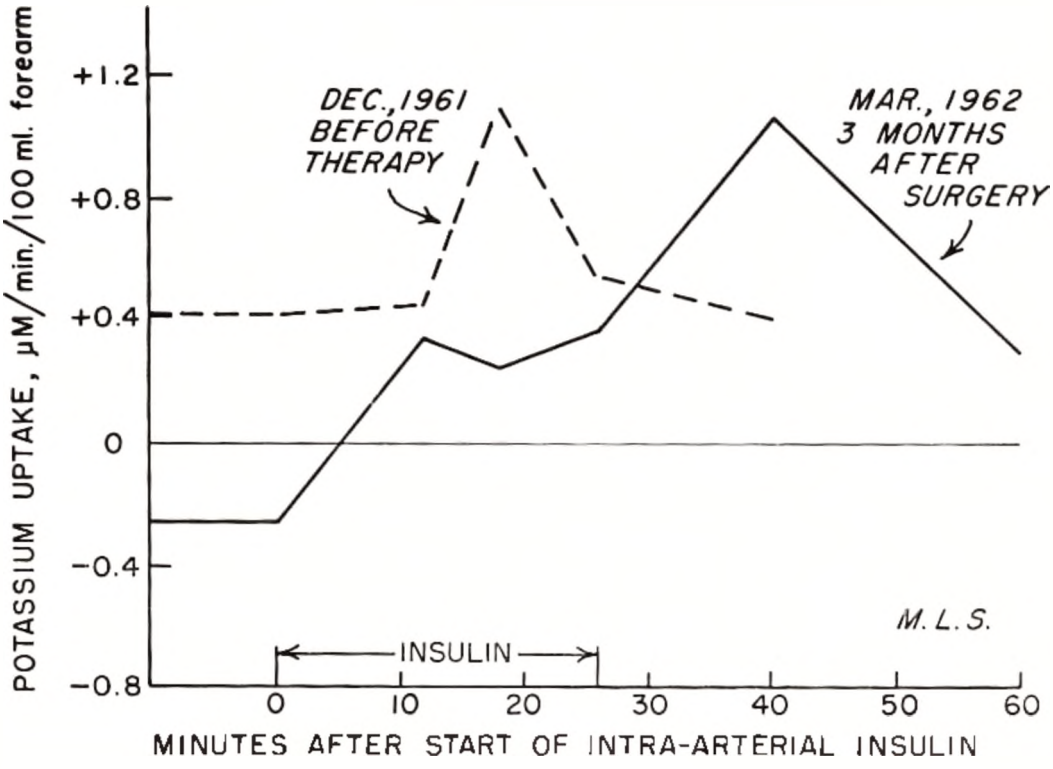

POTASSIUM UPTAKE, μM/min./100 ml. forearm
+1.2
+0.8
+0.4
0
-0.4
-0.8
DEC., 1961
BEFORE
THERAPY
MAR., 1962
3 MONTHS
AFTER
SURGERY
M. L. S.
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 7.

CHAPTER VI

FOREARM METABOLISM IN MAN DURING STARVATION

The effects of starvation on intermediary metabolism in total man have been extensively studied (1, 2). The development by Zierler et al (3) of methods for appraisal of muscle and adipose tissue metabolism in situ has made possible sensitive re-examination of metabolic events provoked by acute starvation in healthy young subjects. The main questions to which answers were sought were: (1) What is the quantitative role of carbohydrate and lipid, in particular, the free fatty acid (FFA) fraction of plasma lipid, in forearm metabolism under basal conditions 66 hours post cibum? and (2) What is the relationship between arterial concentration of various metabolites, such as glucose, and their uptake by forearm muscle?

METHODS

Four young healthy subjects were studied. All received a diet containing 200 g carbohydrate for the three days preceding the test. Catheterisation of the brachial artery and a draining deep and superficial forearm vein was performed on the following morning (Day I) by techniques previously described (3). Blood flow was measured by indicator-dilution techniques based on continuous infusion of the dye T-1824, into the ipsilateral brachial artery. Arterio-deep venous concentrations reflect the metabolism of forearm muscle in the main; arterio-superficial venous concentration differences reflect the metabolism of forearm adipose tissue in the main. At least three sets of observations on basal

forearm metabolism were made. The four subjects, who were kept on the metabolic ward, were allowed water ad lib; a modest amount of exercise was permitted. On the morning of Day III, 48 hours after the first test, and 60-66 hours post cibum, observations on forearm metabolism were repeated employing the same vessels previously catheterised.

RESULTS AND DISCUSSION

Basal forearm muscle metabolism

Results appear in Table I.

Glucose: Glucose uptake by forearm muscle was not different on Day I and Day III of the study, despite a fall of between 20 and 30 per cent in arterial glucose concentration. No correlation was found between arterial concentration and glucose uptake by forearm muscle (Fig. 1). Andres and Zierler had previously shown that direct infusion of glucose into the ipsilateral brachial artery, sufficient to raise the local concentration to 200 mg per cent, did not increase glucose uptake by forearm muscle (4). The present study provides evidence that glucose uptake by muscle remains equally stable in the presence of considerable reduction in arterial glucose concentration. These observations are contrary, however, to the many previous reports describing a "decrease in peripheral glucose utilisation" in starvation (5, 6).

Lactate: No significant change in arterial lactate concentration occurred between the two periods of study. Lactate produc-

tion by forearm muscle on Day III showed a highly significant twofold increase over values obtained on Day I (Fig. 2).

L/G ratio: The expression $\frac{1}{2}$(DV - A lactate difference)/(A - DV glucose difference) yields the fraction of glucose uptake accounted for by lactate production, when concentrations are expressed in moles. This value increased from a mean value of 0.4 on Day I to a mean value of 1.1 on Day III, implying that all glucose uptake was accounted for by lactate production.

Thus, while no temporal trend was observed in glucose uptake by forearm muscle, it was possible, after 66 hours of starvation to account quantitatively for the glucose abstracted from arterial blood by lactate production. Calculation of L/G ratio is based on the assumption that muscle glycogen does not contribute significantly to lactate production. Reasons which point to the probable inadequacy of muscle glycogen as an important substrate have been treated in detail elsewhere (3). In brief, they are based on data of glycogen turnover rates (7), and from observations on decrements in concentration of muscle glycogen (8).

O_2, CO_2: No temporal trend was observed in arterial O_2 concentrations, forearm muscle $\dot{Q}_{O_2}$, and forearm muscle $\dot{Q}_{CO_2}$. In the presence of moderate ketonemia, a modest fall in arterial

CO_2 was recorded on Day III.

G-L/O_2: When lactate production is subtracted from glucose uptake, and the remaining glucose is assumed to have been oxidized, an estimate can be made of the fraction of O_2 uptake accounted for by oxidation of glucose. This fraction decreased from a mean value of 0.18 on Day I to -0.06 on Day III (Fig. 3).

Respiratory Quotient (R.Q): The mean value of forearm muscle R.Q., which was 0.79 on Day I, fell to a mean value of 0.69 on Day III.

Potassium: Escape of potassium from forearm muscle into venous plasma was a consistent feature on Day I and Day III. Arterial potassium concentration remained constant, and there was no temporal trend in potassium output from forearm muscle.

FFA: A - DV FFA concentration differences, which did not differ significantly from zero on Day I, were positive in all four subjects after 66 hours of starvation. This was associated with an increase in arterial FFA concentration, the magnitude of which showed considerable variation.

FFA/O_2: The percentage of O_2 uptake which can be accounted for by complete combustion of FFA abstracted from arterial plasma is given by the following equation:

$$FFA/O_2 = \frac{25(A - DV_{FFA}) \times \text{plasmacrit}}{A - DV_{O_2}}$$

In all four subjects FFA/O_2 ratios of 0.35 or greater were obtained on Day III (Fig. 4); that is, more than one-third of forearm O_2 consumption was accounted for by FFA uptake.

A - SV concentration differences:

Results appear in Table II.

Glucose: Arterio-superficial venous glucose concentration differences obtained on Day III were smaller than values obtained in the immediate postabsorptive period. A degree of correlation was observed between arterial glucose concentration and A - SV concentration differences. This contrasts with the absence of similar correlation in the deep forearm drainage bed.

Lactate: No differences were observed in lactate SV - A concentration differences on Day I and Day III. The rise in L/G ratio was not as great as that observed in the deep forearm drainage bed.

FFA: Negative A - SV FFA concentration differences showed an increase of approximately 50 per cent on Day III, compared with values obtained in the postabsorptive period; that is, they became more negative.

Possible differences in metabolic response to starvation of deep (muscle) and superficial (adipose tissue) forearm drainage beds

Whereas glucose A - DV concentration differences, (and forearm muscle uptake) do not fall with starvation, a good degree of correlation exists between arterial concentration and A - SV glucose differences. Also, whereas lactate DV - A differences show a two-

fold increase 66 hours post cibum, lactate SV - A differences remain unchanged. These observations suggest that the pattern of metabolic events produced by starvation may differ in the deep and superficial forearm drainage beds. We are however unable to measure blood flow through the forearm adipose tissue, and these conclusions are based on the assumption that this was constant on Day I and Day III. A twofold variation in adipose tissue blood flow would render these conclusions spurious.

Possible importance of lactate production in body economy

It has been pointed out previously that lactate production by forearm muscle occurs under resting conditions in the face of vigorous O_2 uptake (3). Under conditions of total starvation, lactate production by forearm muscle shows a twofold increase without any change in either arterial lactate concentration or forearm muscle oxygen consumption. If lactate contributed by forearm muscle is typical quantitatively of total body muscle, then the total amount thus made available is approximately 320 μM per minute or 160 μM per minute, when expressed as glucose equivalents. Absence of significant arterial hyperlactacidemia during starvation in the face of demonstrable increase in production, implies enhanced rate of removal of lactate from the vascular bed. This is chiefly the province of the liver. Total hepatic glucose made available by this means amounts to 30 mg

per minute; that is, about 30 per cent of glucose released by the liver. The so-called Cori cycle thus makes a significant contribution toward maintaining hepatic glucose output in the face of non-availability from dietary sources. It is obvious, however, that this contribution represents no net gain for the body as long as muscle lactate arises from dissimilation of glucose. It remains puzzling that the forearm resorts so extensively to lactate production, which in terms of energy yield is less efficient than aerobic dissimilation.

Quantitative role of lipid as oxidative substrate

In previously reported studies on basal forearm metabolism, the evidence pointing toward a major role for plasma free fatty acids as oxidative substrates for resting muscle has been reviewed (9). Although FFA is being abstracted from arterial plasma by forearm muscle, adipose tissue, surrounding muscle, adds FFA to venous plasma, thereby obscuring any A - V differences. Insulin, by selectively inhibiting FFA release from adipose tissue unmasks a positive A - DV FFA difference, sufficient to account for about 50 per cent of O_2 consumption of forearm muscle (10).

Positive arterio-deepvenous FFA differences were recorded in each of the four subjects fasted for 66 hours, and yield FFA/O_2 ratios of 0.35 or greater. Administration of insulin elevated FFA/O_2 to 1.0 or greater; that is, under conditions of starvation, muscle combusts FFA almost exclusively (Table III). Total extrahepatic oxidation of FFA increases from 0.1mM per minute under

postabsorptive conditions, to 0.2 mM per minute after 66 hours of starvation.

Some indication that FFA release from adipose tissue is increased under conditions of starvation is provided by examination of negative A - SV FFA concentration differences, which show a 50 per cent increase above postabsorptive values. Inability to measure blood flow through forearm adipose tissue precludes accurate quantification of this increase.

Endocrine changes in starvation

It is probable that changes in endocrine function mediate at least some of the metabolic alterations provoked by acute starvation. Through the courtesy of Drs. S.A. Berson and R.S. Yalow, plasma insulin assays (11) were performed on each of the four subjects, both in the postabsorptive period, and again after 66 hours of starvation. Results appear in Table IV. In each of the four subjects, a significant fall in plasma insulin concentration was recorded on Day III.

Previous studies from this laboratory (12) have demonstrated that human growth hormone, infused into the brachial artery of control subjects, increases FFA release from forearm adipose tissue, and increases FFA uptake by forearm muscle. This is accompanied by a fall in R.Q. of forearm muscle. Some of the metabolic events occurring in the forearm of the fasted individual resemble those in the normal forearm exposed to human growth hormone, and suggest that in starvation there is hypersecretion of growth

hormone. This may be one of many functional changes in endocrine status which follow upon cessation of caloric intake, and facilitate increased FFA release from adipose tissue, and increased FFA combustion by muscle.

This hypothesis was advanced by Young some years ago (13), and gains support from demonstration by Golden, Bondy, Chambers, and Geiger (14) of increase in the active acidophile population in human pituitaries examined in patients who had been starved before death. A number of fat mobilizing substances have been isolated from the pituitary gland (15, 16, 17), and the urine of fasting individuals has been found to contain a pituitary dependent substance which has considerable fat mobilizing properties (15). Some of these substances appear to be distinct from human growth hormone, and it is probable that more than one hormone is released by the pituitary during starvation.

It is obvious, of course, that factors other than H.G.H. hypersecretion are involved in the metabolic response to starvation. For example, glucose uptake by skeletal muscle remains unchanged, despite a demonstrable fall in circulating plasma insulin concentration, and good circumstantial evidence for an increase in circulating human growth hormone. Furthermore, glucose entering skeletal muscle is apparently routed quantitatively toward lactate. Maintenance of an adequate Cori cycle

is said to be dependent upon normal adreno-cortical secretion (18). Two recent studies (19, 20) have failed to demonstrate any change in adrenal function during early starvation. It is possible that the observed changes in glucose and lactate metabolism represent a subtle change of muscle membrane whereby permeability to both these substances is increased.

SUMMARY

Basal forearm metabolism has been appraised in four healthy young subjects, fasted for 66 hours. Comparison with metabolic events observed in the postabsorptive state reveals that (1) Glucose uptake by forearm muscle remains unchanged, despite a 20 per cent or greater fall in arterial glucose concentration. (2) Lactate production by forearm muscle increases twofold. Lactate thus quantitatively accounts for glucose uptake by forearm muscle. (3) FFA uptake by forearm muscle is sufficient to account for more than 35 per cent of its oxygen consumption, increasing to 100 per cent following insulin administration. (4) FFA output by adipose tissue (insofar as A - SV differences reflect this) is increased by at least 50 per cent. (5) No temporal trends are observed in $\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, and $\dot{Q}_K$. The concentration of circulating plasma insulin, measured by immunoassay, falls during starvation. Some of the metabolic events observed during starvation suggest that endogenous growth hormone secretion is increased.

is said to be dependent upon normal adreno-cortical secretion (18). Two recent studies (19, 20) have failed to demonstrate any change in adrenal function during early starvation. It is possible that the observed changes in glucose and lactate metabolism represent a subtle change of muscle membrane whereby permeability to both these substances is increased.

SUMMARY

Basal forearm metabolism has been appraised in four healthy young subjects, fasted for 66 hours. Comparison with metabolic events observed in the postabsorptive state reveals that (1) Glucose uptake by forearm muscle remains unchanged, despite a 20 per cent or greater fall in arterial glucose concentration. (2) Lactate production by forearm muscle increases twofold. Lactate thus quantitatively accounts for glucose uptake by forearm muscle. (3) FFA uptake by forearm muscle is sufficient to account for more than 35 per cent of its oxygen consumption, increasing to 100 per cent following insulin administration. (4) FFA output by adipose tissue (insofar as A - SV differences reflect this) is increased by at least 50 per cent. (5) No temporal trends are observed in $\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, and $\dot{Q}_K$. The concentration of circulating plasma insulin, measured by immunoassay, falls during starvation. Some of the metabolic events observed during starvation suggest that endogenous growth hormone secretion is increased.

REFERENCES

1. Benedict, F.G. Study of prolonged fasting. Carnegie Inst. Wash. Publ. No. 203: 1915.
2. Chambers, W.H., Chandler, J.P., and Barker, S.B. The metabolism of carbohydrate and protein during prolonged fasting. J. Biol. Chem. 1939, 131, 95.
3. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.
4. Andres, R., and Zierler, K.L. Stability of glucose uptake by human forearm despite increased arterial glucose concentration. Fed. Proc. 1958, 17, 11.
5. The Physiological Basis of Medical Practice. Best, C.H., and Taylor, N.B. Chapter 48, Williams and Wilkins Co., Baltimore, 1961.
6. Fritz, I.B. Factors influencing the rates of long chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev. 1961, 41, 52.
7. Stetten, De W., Jr. and Boxer, G.E. Studies in carbohydrate metabolism, I. The rate of turnover of liver and of carcass glycogen, studied with the aid of deuterium. J. Biol. Chem. 1944, 155, 231.

8. Hershey, J.M., and Orr, M.D. The removal of glycogen from living muscle. Trans. Roy. Soc. Canada, (Sect. V, Biol. Sc.), 1928, 22, 151.

9. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.

10. Rabinowitz, D., and Zierler, K.L. The effect of insulin on free fatty acid metabolism in the human forearm. Fed. Proc. 1962, 21, 200.

11. Yalow, R.S., and Berson, S.A. Immunoassay of endogenous plasma insulin in man. J. clin. Invest. 1960, 39, 1157.

12. Rabinowitz, D., and Zierler, K.L. Unpublished observations.

13. Young, F.G. Growth and diabetes in normal animals treated with pituitary (anterior lobe) diabetogenic extract. Biochem. J. 1945, 39, 515.

14. Golden, A., Bondy, P,K., Chambers, R.W., and Geiger, C.L. Adenohypophyseal changes in patients dying of neoplastic disease. Yale J. Biol. and Med., 1961, 33, 299.

15. Chalmers, T.M., Pawan, G.L.S., and Kekwick, A. Fat-mobilizing and ketogenic activity of urine extracts. Lancet, 1961, ii, 6.

16. Rudman, D., Reid, M.B., Seidman, F., di Gilolamo, M., Wertheim, A.R., and Bern, S. Purification and properties of a component of the pituitary gland which produces lipemia in the rabbit. Endocrinology, 1961, 68, 273.

17. Seifter, J. and Baeder, D.H. Lipide mobilizer (L.M.) from posterior pituitary of hogs. Proc. Soc. Exptl. Biol. Med. 1957, 95, 318.

18. Long, C.N.H., Smith, O.K., and Fry, E.G. Actions of cortisol and related compounds on carbohydrate and protein metabolism. Ciba Foundation Study Group, No. 6. Wolstenholme, G.E.W., and O'Connor, M., Eds. Little, Brown and Company, 1960, p. 4.

19. Wood, F.C., Jr., Domenge, L., Bally, P.R., Renold, A.E., and Thorn, G.W. Studies on the metabolic response to prolonged fasting. Medical Clinics of North America. 1960, 44, 1371.

20. Slater, G.G., Doctor, R.F., and Kollar, E.J. Plasma and Urinary corticoids of fasting men. Forty-fourth meeting of the Endocrine Society abstract 46, p. 23, June, 1962.

Table I

Forearm Metabolism in the Postabsorptive State and After 66 Hours Starvation*

Oxygen

Subject	Day I			Day III		
	A	A-DV	Q	A	A-DV	Q
F	8.80	2.98	10.63	8.86	1.47	8.27
S	7.13	4.02	7.87	6.82	2.40	6.68
K	9.26	2.05	9.97	8.82	2.92	13.94
C	8.02	2.83	9.68	7.80	1.40	6.17
Mean	8.30	2.97	9.54	8.10	2.05	8.78

Glucose

Subject	Day I			Day III		
	A	A-DV	Q	A	A-DV	Q
F	4.38	0.08	0.28	3.32	0.05	0.28
S	4.40	0.21	0.41	3.23	0.11	0.33
K	4.94	0.08	0.30	3.74	0.13	0.59
C	5.20	0.10	0.43	3.72	0.10	0.36
Mean	4.73	0.12	0.36	3.50	0.10	0.39

FFA

Subject	Day I		Day III	
	A	A-DV	A	A-DV
F	0.95	-0.08	1.43	+0.06
S	0.87	-0.03	0.99	+0.09
K	0.63	-0.03	1.44	+0.12
C	0.82	+0.02	1.89	+0.04
Mean	0.82	-0.03	1.44	+0.08

Potassium

Subject	Day I			Day III		
	A	A-DV	Q	A	A-DV	Q
F	3.90	-0.16	-0.28	4.01	-0.02	-0.05
S	3.97	-0.20	-0.25	4.05	-0.06	-0.13
K	3.95	-0.11	-0.29	3.71	-0.16	-0.44
C	3.77	-0.04	-0.09	3.83	-0.20	-0.49
Mean	3.90	-0.13	-0.23	3.90	-0.11	-0.28

Table I (continued)

Forearm Metabolism in the Postabsorptive State and After 66 Hours Starvation

CO_2

Subject	Day I			Day III		
	A	A-DV	Q	A	A-DV	Q
F	19.48	2.26	8.07	17.81	0.75	4.22
S	20.40	2.32	4.57	17.70	1.46	4.06
K	21.88	1.87	9.10	19.24	2.35	11.22
C	20.79	2.64	0.01	15.66	1.01	4.44
Mean	20.64	2.25	7.69	17.60	1.39	5.99

Lactate

Subject	Day I			Day III		
	A	A-DV	Q	A	A-DV	Q
F	0.65	-0.09	-0.17	0.69	-0.17	-0.98
S	0.53	-0.21	-0.42	0.54	-0.21	-0.62
K	0.60	-0.14	-0.66	0.62	-0.21	-0.95
C	0.65	-0.10	-0.37	0.77	-0.28	-1.09
Mean	0.60	-0.14	-0.40	0.65	-0.22	-0.91

R.Q.

Subject	Day I	Day III
F	0.76	0.51
S	0.58	0.71
K	0.91	0.80
C	0.93	0.72
Mean	0.79	0.69

Plasma Flow

Subject	Day I	Day III
F	1.7	3.1
S	1.2	1.9
K	2.6	2.6
C	4.3	2.3
Mean	2.46	2.48

*Day I and Day III refer to studies performed 12 hours and 66 hours post cibum respectively. Units: Plasma flow, ml/min·100 ml forearm; A = arterial concentration, μM/ml whole blood (O_2, CO_2, glucose and lactate) or plasma (Potassium, FFA); (A-DV) = arterio-deep venous concentration difference, μM/ml; Q = uptake or outflow, μM/min/100 ml forearm.

Table II

Glucose, Lactate and FFA A-SV Differences

12 Hours (Day I) and 66 Hours (Day III) Post Cibum

Glucose. (A-SV) Differences

μM/ml

Subject	Day I	Day III
F	0.16	0.08
S	0.32	0.13
K	0.22	0.19
C	0.23	0.16
Mean	0.23	0.14

Lactate, (A-SV) Differences

μM/ml

F	-0.15	-0.10
S	-0.24	-0.16
K	-0.17	-0.20
C	-0.25	-0.27
Mean	-0.20	-0.18

FFA. (A-SV) Differences

μM/ml

F	-0.11	-0.15
S	-0.20	-0.21
K	-0.11	-0.19
C	-0.14	-0.20
Mean	-0.14	-0.19

Table III

Effect of Insulin on A-DV FFA Differences and FFA/O_2 in Subjects Starved for 66 Hours

Subject	Basal (66 hours post cibum)		Peak Insulin Effect	
	(A-DV)	FFA/O_2	(A-DV)	FFA/O_2
S	+0.08	0.34	+0.24	1.44
K	+0.12	0.38	+0.20	1.02
C	+0.04	0.27	+0.31	1.45

Arterio-deep venous FFA concentration differences and FFA/O_2 ratios, under basal conditions 66 hours post cibum, and following local intra-arterial insulin administration (100 μU/kg/min for 26 minutes). Since insulin inhibits FFA release from forearm adipose tissue but does not directly influence the net uptake of FFA by peripheral tissues, it serves to unmask the quantitative role of FFA in forearm metabolism (see text).

Table IV

Brachial Arterial Plasma Insulin Concentrations

Subject	Day I	Day III
F	9.0	0
S	11.5	8.0
K	5.0	1.5
C	10.5	3.5
Mean	9.0	3.0

Arterial plasma insulin concentrations 12 hours (Day I) and 66 hours (Day III) post cibum. Assays performed by Drs. S. A. Berson and R. S. Yalow by their immuno-assay technique (11). All values expressed as μU/ml.

LEGEND TO FIGURES

Fig. 1. Lack of correlation between arterial glucose concentration and glucose uptake by forearm muscle. Each subject is identified by an initial. Subscripts 1 and 2 refer to observations on Day I and Day III of the study.

Fig. 2. Comparison of lactate production in each of the four subjects on Day I and Day III of the study.

Fig. 3. Change in G-L/O_2 ratio with starvation.
G-L/O_2 = fraction of O_2 consumption accounted for by glucose oxidation.

Fig. 4. Change in apparent FFA/O_2 with starvation.
FFA/O_2 = fraction of O_2 consumption accounted for by FFA oxidation.

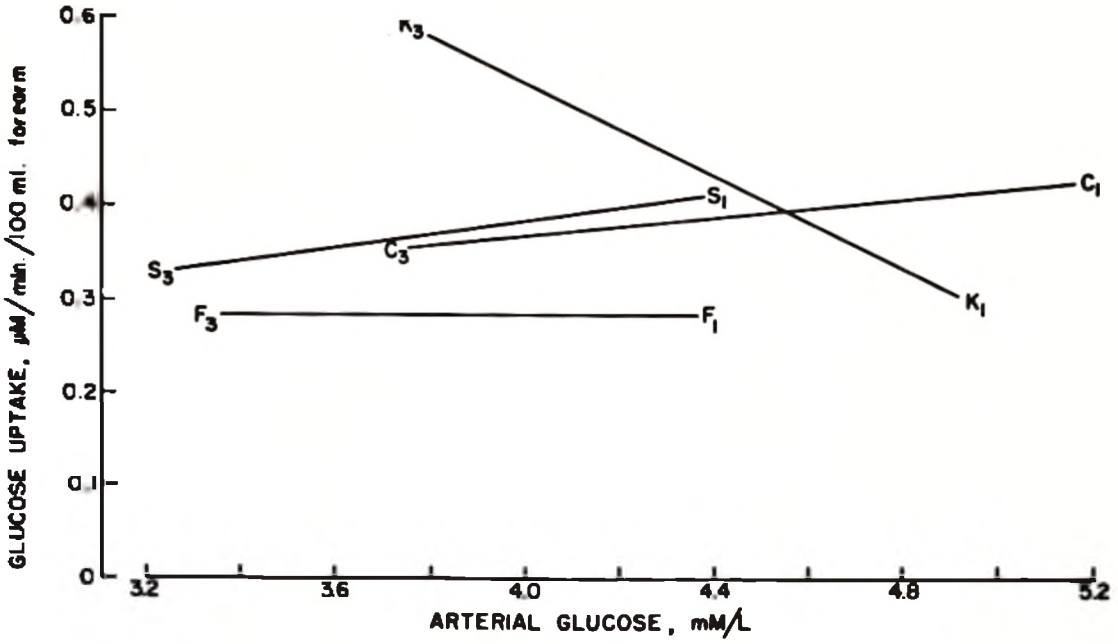

GLUCOSE UPTAKE, μM/min./100 ml. forearm
0.6
0.5
0.4
0.3
0.2
0.1
0
K3
S1
C1
S3
C3
K1
F3
F1
3.2
3.6
4.0
4.4
4.8
5.2
ARTERIAL GLUCOSE, mM/L

FIG. 1

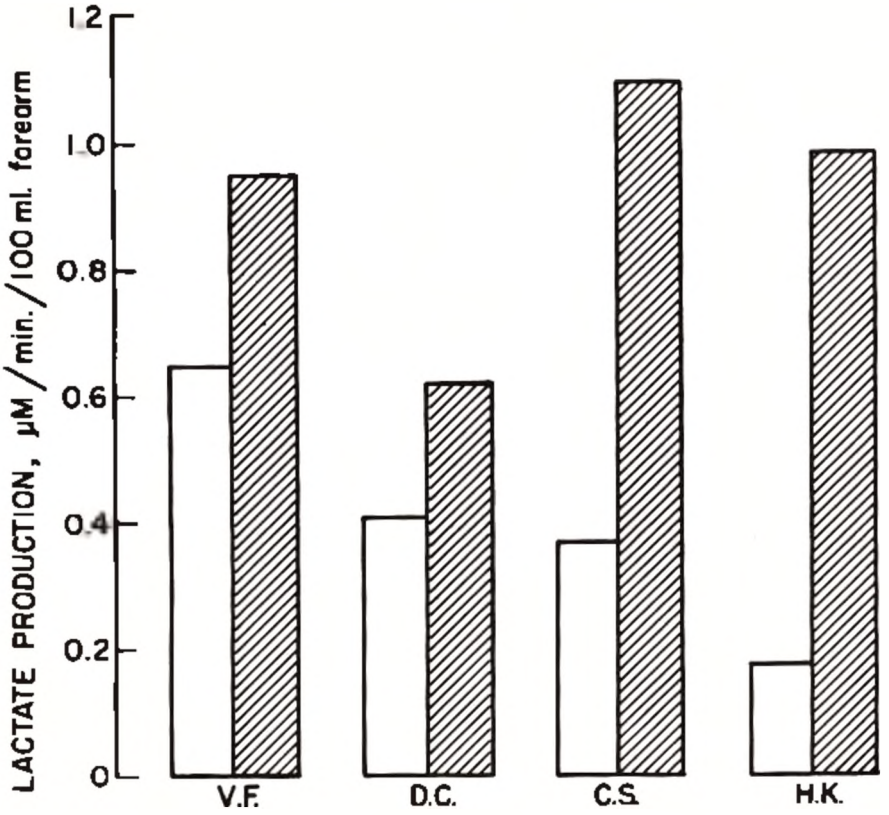

LACTATE PRODUCTION, μM/min./100 ml. forearm
1.2
1.0
0.8
0.6
0.4
0.2
0
V.F.
D.C.
C.S.
H.K.

FIG 2.

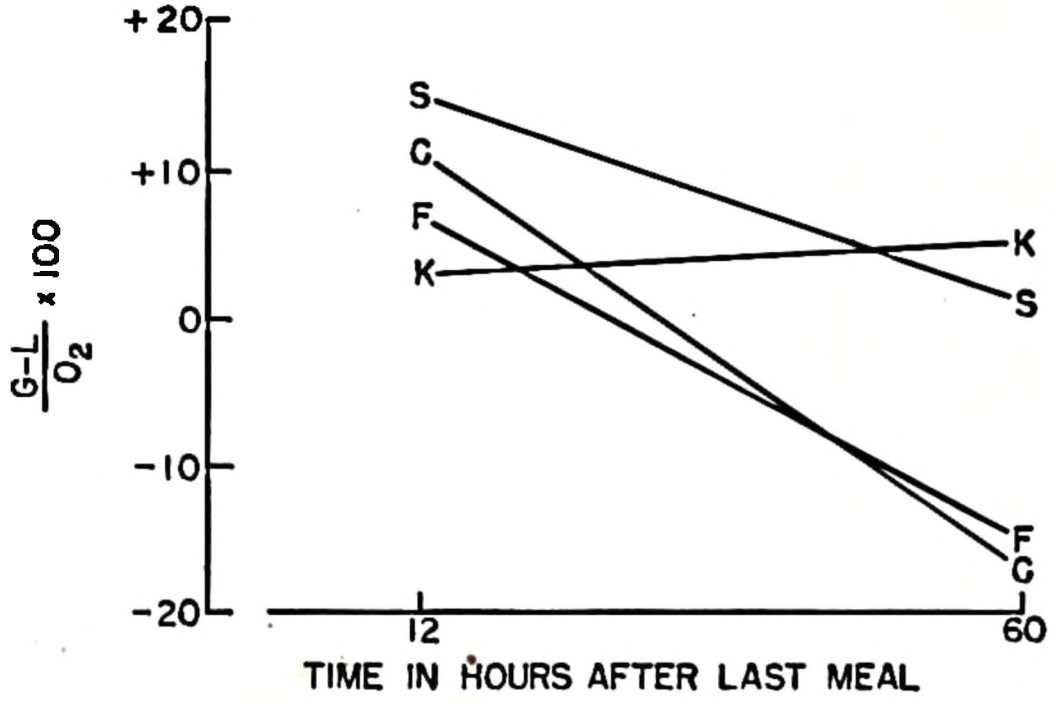

+20
+10
0
-10
-20
$\frac{G-L}{O_2} \times 100$
S
C
F
K
K
S
F
C
12
60
TIME IN HOURS AFTER LAST MEAL

FIG. 3.

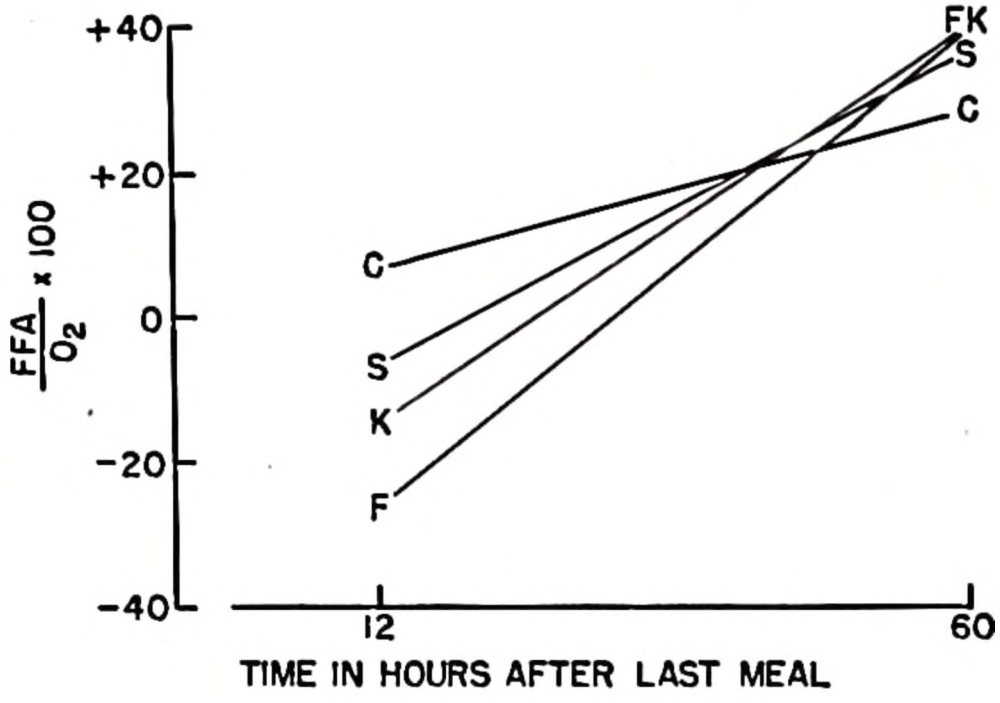

+40
+20
0
-20
-40
$\frac{FFA}{O_2} \times 100$
FK
S
C
C
S
K
F
12
60
TIME IN HOURS AFTER LAST MEAL

FIG. 4.

CHAPTER VII

DISSOCIATION OF INSULIN ACTION IN THE FOREARM OF MAN

Effects of a standard dose of insulin, administered by close intra-arterial injection, on forearm muscle and adipose tissue metabolism have previously been quantified in young healthy control subjects (1, 2). The phenomenon of so-called "insulin resistance" is common to a variety of circumstances, and the literature is replete with observations pointing to resistance to the hypoglycemic effects of exogenous insulin in starvation, Cushing's disease and acromegaly (3, 4, 5). Few studies, however, have reported the responsiveness to insulin in these conditions, with respect to other metabolic effects. This communication reports our observations on dissociation of insulin action in the forearm of man, which appears to be common to all three situations: Insulin-induced glucose and potassium uptake by muscle and, to a lesser extent, by adipose tissue was demonstrably inhibited, but insulin-induced inhibition of FFA release from adipose tissue was either unchecked, or in some cases enhanced.

METHODS

Studies were performed on six patients with active acromegaly, three patients with Cushing's syndrome, and three normal subjects in whom endogenous growth hormone was stimulated by a 66-hour fast. Subjects with acromegaly and Cushing's syndrome were studied between the hours of 9 A.M. and 1 P.M., that is, 13-17 hours after their last meal. Dietary history of carbohydrate intake was normal. The brachial artery, an ipsilateral

deep vein (DV) draining chiefly muscle and an ipsilateral superficial vein (SV) draining chiefly skin and adipose tissue were catheterised by techniques described previously (6). Either two or three sets of control samples were taken. Blood flow was measured by indicator-dilution techniques, based on continuous infusion of the dye T-1824. Insulin was then administered by continuous infusion for 26 minutes at a rate that delivered 100 μU of insulin per minute per kg body weight. This dose, while large enough to exert measurable local effects does not, upon dilution in total blood volume, produce any systemic effects in control subjects (1). Blood samples were drawn 12, 18 and 26 minutes after the start of intra-arterial insulin and for periods up to one hour thereafter. Blood flow to the hand was occluded by a sphygmomanometer cuff about the wrist, inflated to more than 250 mm Hg pressure, during and for at least 5 minutes preceding collection of blood samples, and for the entire 26 minutes of insulin administration. Net uptake (or output) of a metabolite by forearm tissues, chiefly skeletal muscle, was calculated by the equation $\dot{Q} = F(A - DV)$ where $\dot{Q}$ is uptake (or output) of the metabolite in units of mass per minute per 100 ml. of forearm, F is blood (or plasma) flow through the forearm in units of ml per minute per 100 ml. of forearm, and A and DV are concentrations of the metabolite in arterial and deep venous blood (or plasma) respectively, in units of mass per ml. The errors have been discussed elsewhere (7).

RESULTS

Effect of insulin on metabolism of DV drainage bed in acromegaly. Cushing's syndrome and starvation.

Results appear in Table I.

Glucose: Glucose uptake in response to insulin by tissue drained by the DV (chiefly skeletal muscle) was reduced to approximately one half that seen in control subjects (Fig. 1). In subjects with Cushing's syndrome, there was unequivocal evidence of antagonism to the action of insulin on glucose uptake by peripheral tissues, a feature of adrenal hypercorticism which has previously often been disputed (8).

Lactate: There was a small but definite increase in lactate production after insulin administration. The fraction of glucose uptake accounted for by lactate production (the L/G ratio) decreased during and after insulin administration from 0.39 to 0.17 in acromegaly, from 0.61 to 0.32 in Cushing's syndrome and from 1.01 to 0.55 in starvation.

Potassium: Insulin-induced net inward movement of potassium into forearm was less in the three abnormal groups than in controls. The degree of inhibition of insulin effect was perhaps greater in acromegaly than in starvation and Cushing's disease (Fig. 2). There were, of course, considerable differences in basal potassium movement across the forearm among the three groups: In starvation, as in controls, K^+ moved out of forearm into venous plasma; In Cushing's syndrome, escape of K^+ from

forearm was considerably exaggerated; In acromegaly there was net inward movement of K^+ into forearm in the basal state.

Effect of insulin on A - SV concentration differences.

Results appear in Table II.

Glucose: Each of the three abnormal groups exhibited moderate inhibition of insulin-induced glucose uptake by forearm tissue drained by SV, insofar as this is reflected by A - SV concentration differences (Fig. 3). The degree of inhibition displayed, certainly with respect to the acromegalic subjects studied, was of lesser magnitude than that evidenced in the deep forearm bed.

Lactate: A small increase in negative A - SV lactate differences occurred after insulin, with a corresponding modest fall in L/G ratio.

Potassium: A moderate degree of inhibition in insulin-induced change in K^+ A - SV concentration differences was noted (Fig. 4).

Free fatty acids (FFA): Administration of insulin in the experimental groups, as in controls, effectively inhibited net release of FFA from forearm adipose tissue; (Figs. 5, 6 and 7), that is, A - SV FFA concentration differences, negative under basal conditions, were reduced to zero or became positive with insulin. Since basal negative A - SV FFA concentration differences were greater (more negative) in starvation, acromegaly and Cushing's disease than in controls, the net change

in A - SV FFA concentration difference following insulin was at least equal to that found in control subjects. In acromegalic subjects, who exhibit a threefold increase in basal negative A - SV FFA differences, the insulin-induced change in A - SV FFA was greater than in controls (Fig. 5). The 66-hour fasted subjects were so exquisitely sensitive to the hypolipacidemic effects of insulin that arterial FFA concentrations fell by 20 per cent or more following insulin administration (Fig. 6). Since there is a 100-fold dilution of the local concentration of insulin (about 300 μU insulin per ml. brachial arterial plasma) in total blood volume, observed changes in arterial FFA were in response to an estimated maximum concentration of insulin of 3 μU per ml. recirculating plasma.

DISCUSSION

There have been reports from time to time indicating that plasma, or various plasma fractions may exhibit little or no insulin-like activity (I.L.A.) when exposed to excised rat hemi-diaphragm, and yet retain good insulin-like activity when exposed to excised rat adipose tissue. For example, Steinke, Taylor and Renold have reported that plasma taken from untreated juvenile diabetic subjects exhibits normal I.L.A., when this is measured by the CO_2 production which follows incubation of excised adipose tissue with plasma, but very low I.L.A., as measured by the ability of the plasma to increase glucose uptake by rat hemidiaphragm (9).

Similarly, the observations of Vallance-Owen and Lilley (10), and those of Lowy, Blanshard and Phear (11) suggest the presence in albumin fractions of normal human sera of an inhibitor to insulin-induced glucose uptake by isolated rat diaphragm; this albumin fraction does not, however, depress insulin-induced glucose uptake or CO_2 production by the epididymal pad of fat. It has been proposed, therefore, that under the proper circumstances, there may be inhibition of insulin action with respect to muscle but not to fat (10).

There is thus nothing novel in the suggestion that certain actions of insulin may be either selectively preserved, or selectively depressed. In patients with hypophyseal-adrenocortical disease, there was the anticipated poor response to insulin with respect to peripheral glucose and potassium movement, but insulin-induced inhibition of FFA release from adipose tissue was retained.

This may indicate either (1) discrimination of insulin action between different tissues (for example, selective inhibition on muscle, but not on fat) or (2) discrimination between various insulin-dependent metabolic reactions (for example, inhibition of insulin-induced glucose and potassium movement into both muscle and fat, but unchecked insulin action on FFA movement in fat). While the former solution would be in line with much of the in vitro evidence, we do not favor it. In the human forearm, A - DV concentration differences reflect metabolism of muscle in the main, and A - SV differences reflect metabolism of adipose tissue

in the main (6). Therefore, if the action of insulin were selectively inhibited with respect to muscle, then the insulin-induced increase in A - SV differences in hypophyseal-adrenocortical diseases should be the same as controls. This is not so: In all three groups, both A - DV and A - SV glucose and potassium differences following insulin are significantly less than controls (Figs. 1, 2, 3 and 4).

Of course, SV effluent drains not only adipose tissue but also skin, and there is undoubtedly contamination by effluent from the deep forearm bed. We therefore have to consider the possibility that the diminution of response to insulin which is reflected in A - SV differences of glucose and potassium may be the consequence of contamination of SV by effluent from non-adipose tissue, rather than truly indicating resistance of adipose tissue to insulin. Two considerations make this unlikely: FFA arises exclusively from adipose tissue. Since basal negative A - SV FFA differences, which reflect release of FFA from forearm adipose tissue, were greater in all three groups studied than in controls, the contribution of adipose tissue to SV effluent even though not quantifiable was undoubtedly significant. Secondly, to account for the observed reduction in magnitude of insulin-induced glucose and potassium A - SV differences on the basis of DV admixture, would require contamination of SV by an excess of 30 per cent. Because O_2 and CO_2 A - DV differences are considerably greater than the respective

A - SV differences (6), addition to SV effluent of say 30 per cent DV blood would cause a measurable increase in A - SV differences of these gases. No such increase was observed.

For these reasons, the data are more consonant with the notion that the action of insulin on both potassium and glucose uptake peripherally is discriminated from its action on FFA release from adipose tissue (Fig. 8).

It is pertinent to inquire what the various conditions which provoke dissociation of insulin action may have in common. In all, there is disturbance of the hypophyseal-adrenocortical axis. In both acromegaly and acute starvation, human growth hormone secretion is increased (12, 13), and furthermore, dissociation of insulin action in the forearm may be produced acutely by simultaneous HGH administration (14). How may the presence of increased circulating HGH, and perhaps circulating cortisol, modify insulin action in this fashion?

Let us consider the hypothesis that the locus of insulin action is at the cell membrane. It has been proposed, from measurement of change in electrical potential across mammalian muscle fibre membranes, that a change in the fixed electrical charge within the membrane is produced by the association of insulin and the membrane, and that insulin, by hypothetically deforming the membrane, increases its permeability (15). In the light of recent demonstration by Beigelman and Hollander (16) that insulin also increases electrical potential of isolated adipose tissue cells, this hypothesis may also hold true for adipose tissue.

Circulating antagonists, such as HGH, may act (1) by preventing the normal association between insulin and the membrane, thereby leaving cell permeability to glucose unaltered or (2) by forming a complex with insulin, and now the hypothetical association of the HGH-insulin complex with the membrane, may render the new membrane poorly permeable to glucose.

If the postulate is true that insulin induces glucose and potassium entry into peripheral cells by changing the cell membrane, then inhibition of these effects, with preservation of insulin effect on FFA release, suggests discrete loci of insulin action, and makes any unitary hypothesis of insulin action inadequate.

To a degree this interpretation runs contrary to the generally held view that insulin-induced inhibition of FFA release from adipose tissue is secondary to enhanced glucose uptake by the tissue, which increases intracellular L-glycerol phosphate, thereby favoring net synthesis of triglyceride (17). Our studies favor the notion that insulin may have a more direct role, possibly intracellularly, in causing inhibition of fatty acid release from adipose tissue. Stimulation of the necessary enzyme systems whereby glucose may be selectively channeled to L-glycerol phosphate is of course a possibility. This may be comparable to the insulin effect in rat diaphragm where, by increasing UDPG glycogen transglucosylase activity, glucose is channeled towards glycogen (18).

These observations then need not contradict the in vitro evidence that the presence of glucose is essential for insulin to reduce FFA release from adipose tissue or that this action is in fact mediated by increasing intracellular esterification of fatty acids (17).

The percentage of glucose taken up under insulin stimulation (less that accounted for by lactate production) which must be diverted into L-glycerol phosphate to that end (the "glyceride-glycerol drain") is given by the following equation:

$$\text{Glyceride-glycerol drain} = \frac{\left[1/6\ (A\text{-}SV)\ FFA \text{ at peak insulin effect} - \text{Basal } (A\text{-}SV)FFA\right] \times \text{plasmacrit}}{(A\text{-}SV)\ \text{Glucose} - \frac{1}{2}(A\text{-}SV)\ \text{lactate at peak insulin effect}}$$

In control subjects, this ratio is less than 0.05; that is, if insulin inhibits FFA release from adipose tissue by favoring its re-esterification, less than 5 per cent of available glucose need be diverted to L-glycerol phosphate to that end. In each of the three abnormal situations studied, the calculated 'glyceride-glycerol drain' is greater than 30 per cent but less than 100 per cent, which certainly suggests preferential channeling of glucose to L-glycerol phosphate.

These studies do not, of course, preclude the possibility that in addition to insulin, the circulating inhibitors, HGH and cortisol in the conditions studied, may also have more than one site of action. Also left unanswered is the question of whether the two hormones are themselves acting as inhibitors, or whether

they are instrumental in provoking the production of an inhibitor or inhibitors. With respect to HGH, however, unpublished experiments from our laboratory indicate that the dissociation of insulin action described above may be observed acutely in the forearm following simultaneous injection of HGH (0.2 μgm/kg/min.) and insulin (100 μU/kg/min.) into the brachial artery.

SUMMARY

The effect of insulin, administered by constant close arterial injection, on metabolism of human forearm in situ was observed in 6 patients with active acromegaly, 3 with Cushing's syndrome and in 3 normal subjects in whom endogenous growth hormone activity was stimulated by a 66-hour fast. Metabolism was assessed by measurement of forearm blood flow and arteriovenous concentration differences. Glucose and potassium uptake by muscle in response to insulin was less than half normal. Insulin-induced glucose and potassium uptake by adipose tissue was reduced, but was probably less affected than muscle uptake. Insulin-induced inhibition of FFA release from adipose tissue was unchecked in these patients. The 66-hour fasted subjects were so sensitive that arterial FFA concentrations fell in response to an estimated maximum 3 μU of insulin/ml recirculating plasma. These studies appear to dissociate the action of insulin on potassium and on glucose uptake from its effect on FFA release. They make a unitary explanation of insulin action difficult.

REFERENCES

1. Andres, R., Baltzan, M.A., Cader, G., and Zierler, K.L. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. J. clin. Invest. 1962, 41, 108.
2. Rabinowitz, D., and Zierler, K.L. Role of FFA in forearm metabolism in man, quantitated by use of insulin. J. clin. Invest. in press.
3. Himsworth, H.P., and Kerr, R.B. Insulin and alimentary hyperglycemia in young normal subjects. Clin. Sci. 1939, 4, 1.
4. Fraser, R., Joplin, G.F., Opie, L.H., and Rabinowitz, D. The augmented insulin tolerance test for detecting insulin resistance. J. Endocrinol. in press.
5. Fraser, T.R.C. Endocrine disorders and insulin action. Brit. med. Bull. 1960, 16, 242.
6. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.
7. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.
8. Long, C.N.H., Smith, O.K., and Fry, E.G. Actions of cortisol and related compounds on carbohydrate and protein metabolism. Ciba Foundation Study Group, No. 6. Wolstenholme, G.E.W., and O'Connor, M., Eds. Little, Brown and Company, 1960, p.4.

9. Steinke, J., Taylor, K.W., and Renold, A.E. Insulin and insulin antagonists in the serum of untreated juvenile diabetes. Lancet, 1961, 1, 30.

10. Vallance-Owen, J., and Lilley, M.D. Insulin antagonism in the plasma of obese diabetics and prediabetics. The Lancet 1961, 1, 806.

11. Lowy, C., Blanshard, G., and Phear, D. Antagonism of insulin by albumin. Lancet, 1961, 1, 802.

12. Golden, A., Bondy, P.K., Chambers, R.W., and Geiger, C.L. Adenohypophyseal changes in patients dying of neoplastic disease. Yale J. Biol. and Med., 1961, 33. 299.

13. Rabinowitz, D., and Zierler, K.L. Unpublished observations.

14. Rabinowitz, D., and Zierler, K.L. Unpublished observations.

15. Zierler, K.L. Effect of insulin on membrane potential and potassium content of rat muscle. Amer. J. Physiol. 1959, 197. 515.

16. Beigelman, P.M., and Hollander, P.B. Response of adipose tissue membrane resting electrical potential to insulin. Fed. Proc. 1962, 21, 205.

17. Bally, P.R., Cahill, G.F., Jr., Leboeuf, B., and Renold, A.E. Studies on rat adipose tissue in vitro. V. Effects of glucose and insulin on the metabolism of palmitate-1-C^{14}. J. Biol. Chem. 1960, 235, 333.

18. Villar-Palasi, C., and Larner, J. Insulin treatment and UDPG glycogen transglucosylase activity. Fed. Proc. 1960, 19, 164.

Table I

Mean Effect of Insulin on Forearm Metabolism in Acromegaly, Cushing's Disease and 66-hour Starvation

Glucose Uptake

μM/min/100 ml arm

Time after insulin started min	Acromegaly	Cushing's Disease	Starvation	Controls
0	0.69	0.52	0.42	0.52±0.075
12	1.05	0.41	0.64	2.70±0.811
18	1.94	0.61	0.69	4.71±1.026
26	1.81	1.60	1.46	6.78±1.20
40	1.97	1.36	0.97	6.07±0.998
60	1.84	0.57	0.71	3.52±0.645

Net Inward Movement of Potassium

μM/min/100 ml arm

0	0	0	0	0
12	-0.15	0.23	0.22	0.62±0.292
18	-0.10	0.39	0.46	1.06±0.367
26	-0.04	0.81	0.83	1.24±0.355
40	+0.12	0.98	0.79	1.47±0.375
60	+0.13	1.00	0.74	1.30±0.294

Insulin administered for 26 minutes, beginning at time zero. Control values are means ±S.E. of means among ten control subjects. Potassium data expressed as change in potassium movement following insulin, compared to potassium movement at time zero.

Table II

Mean Effect of Insulin on A-SV Concentration Differences in Acromegaly, Cushing's Disease and 66-hour Starvation

Glucose, (A-SV) Differences

μM/ml

Time after insulin started min	Acromegaly	Cushing's Disease	Starvation	Controls
0	0.21	0.22	0.16	0.17±0.013
12	0.34	0.09	0.09	0.43±0.048
18	0.39	0.20	0.27	0.65±0.060
26	0.53	0.28	0.40	0.83±0.038
40	0.55	0.36	0.14	0.80±0.032
60	0.48	0.38	0.12	0.50±0.044

Lactate, (A-SV) Differences

μM/ml

0	0.17	0.14	0.21	0.15±0.015
12	0.21	0.13	--	0.12±0.011
18	0.24	0.22	0.26	0.17±0.029
26	0.20	0.15	0.22	0.23±0.030
40	0.28	0.24	0.20	0.21±0.035
60	0.36	0.19	0.24	0.24±0.037

Potassium, (A-SV) Differences

μM/ml

0	+0.05	-0.19	-0.13	-0.04±0.033
12	+0.07	-0.05	-0.12	+0.09±0.050
18	+0.06	-0.06	-0.01	+0.10±0.030
26	+0.13	0.0	+0.04	+0.22±0.040
40	+0.07	0.06	+0.06	+0.22±0.070
60	+0.09	-0.15	+0.05	+0.24±0.040

Insulin administered for 26 minutes, beginning at time zero.

LEGEND TO FIGURES

Fig. 1. Glucose uptake by forearm following intra-arterial insulin in acromegaly, Cushing's syndrome and starvation. All values are means ± S.E. of means.

Fig. 2. Potassium movement into forearm following intra-arterial insulin in acromegaly, Cushing's syndrome and starvation. Data plotted as change in potassium A - DV differences compared to A - DV differences at time zero. All values are means ± S.E. of means.

Fig. 3. Glucose A - SV differences following insulin in acromegaly, Cushing's syndrome and starvation. All values are means ± S.E. of means.

Fig. 4. Change in potassium A - SV differences following insulin in acromegaly, Cushing's syndrome and starvation. All values are means ± S.E. of means.

Fig. 5. A - SV FFA differences before, during and after insulin in active acromegaly. Data are presented as change in A - SV FFA differences, compared with A - SV differences at time zero. Each subject is indicated by an initial. Control values (mean ± S.E. of mean) are also shown.

Fig. 6. Arterial (A) and superficial venous (V) FFA levels before, during and after insulin in three subjects fasted for 66 hours. Mean control values are also shown.

g. 7. Arterial (A) and superficial venous (V) FFA levels before, during and after insulin in two patients with acromegaly and two patients with Cushing's syndrome. Mean control values are also shown.

g. 8. Schematic representation of dissociation of insulin action. Normal actions of insulin in facilitating translocation of glucose and potassium from blood into peripheral tissues, and inhibiting FFA release from adipose tissue, are represented by solid black arrows. Cross-hatched arrows indicate which actions of insulin are inhibited in hypophyseal-adrenocortical disease.

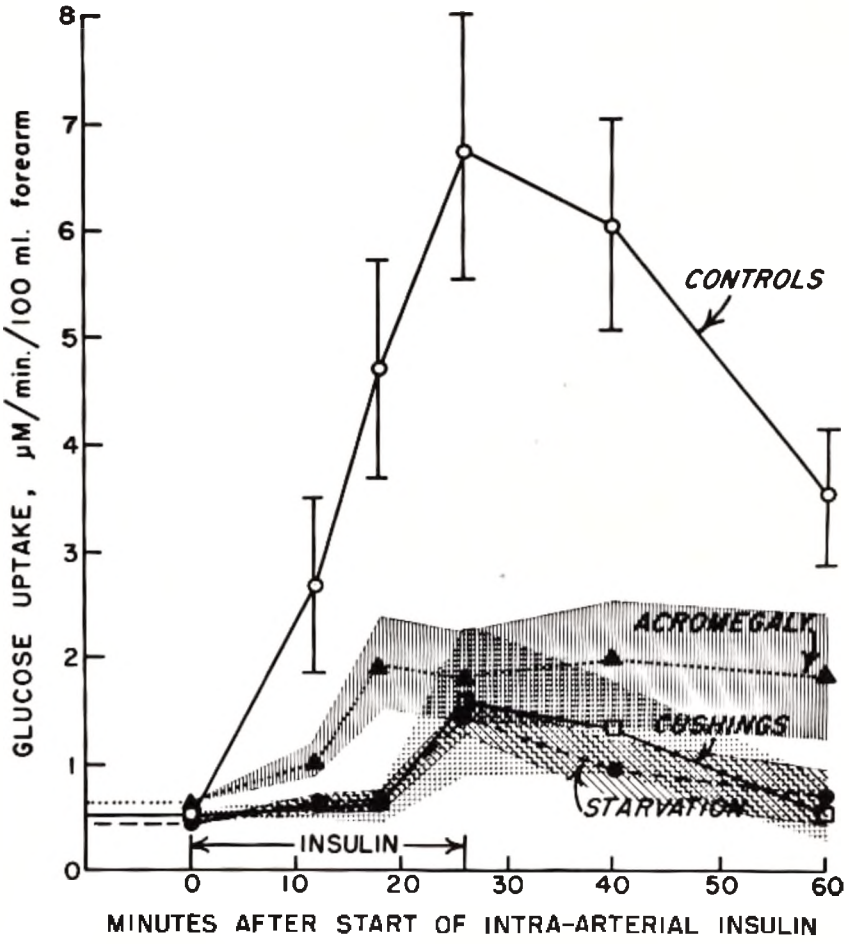

GLUCOSE UPTAKE, μM/min./100 ml. forearm
8
7
6
5
4
3
2
1
0
CONTROLS
ACROMEGALY
CUSHINGS
STARVATION
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 1.

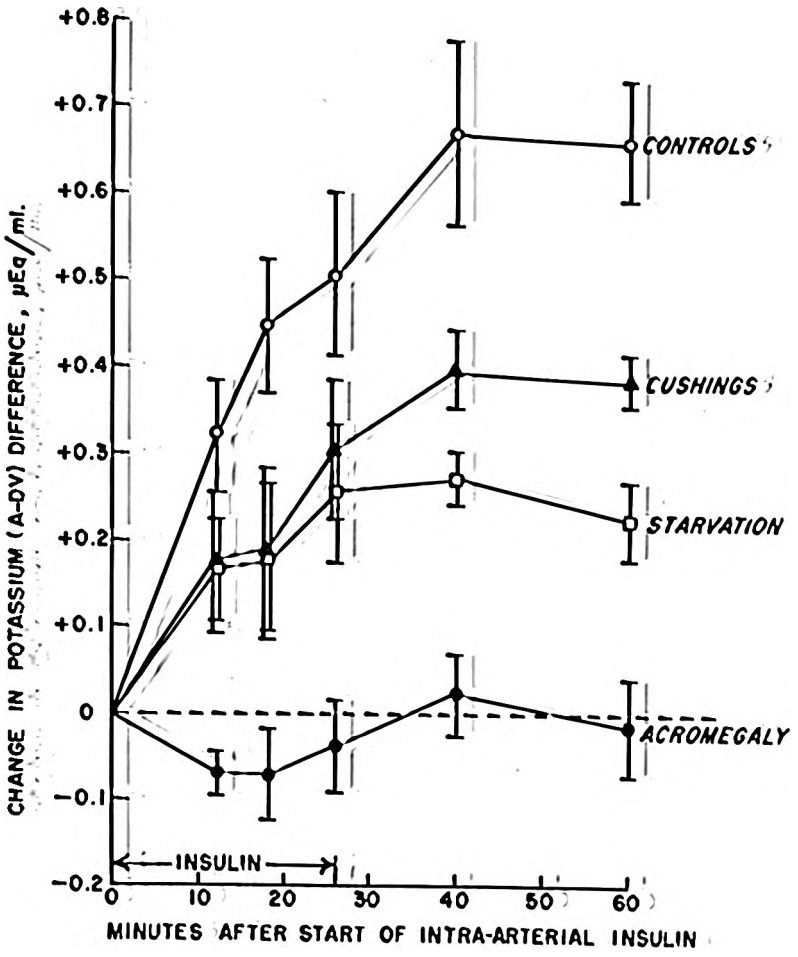

+0.8
+0.7
+0.6
+0.5
+0.4
+0.3
+0.2
+0.1
0
−0.1
−0.2
CHANGE IN POTASSIUM (A-DV) DIFFERENCE, μEq/ml.
CONTROLS
CUSHINGS
STARVATION
ACROMEGALY
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 2.

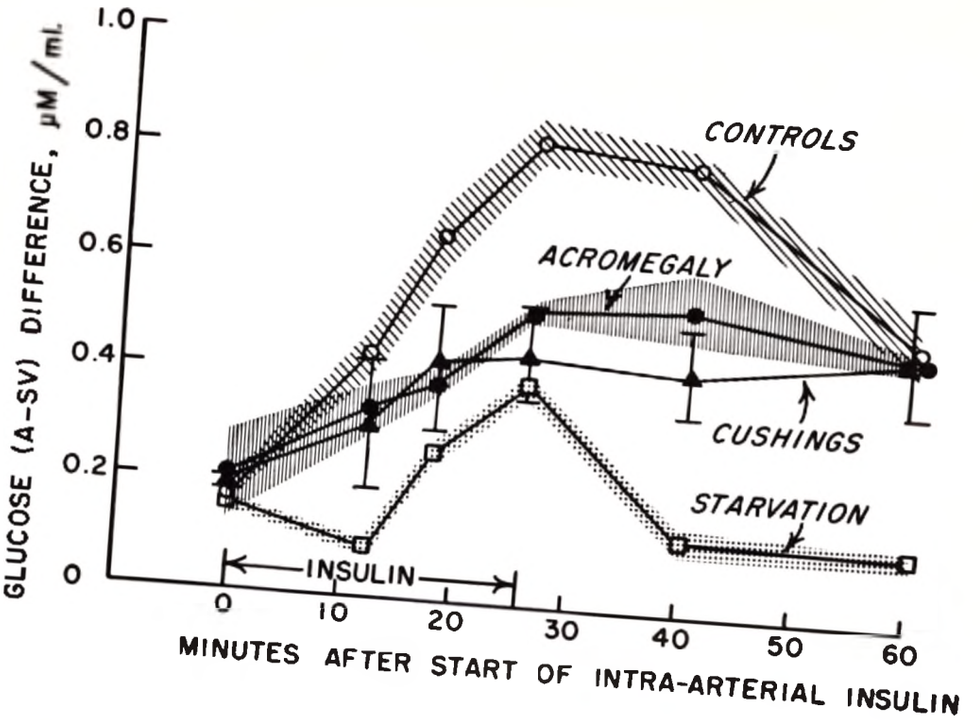

GLUCOSE (A-SV) DIFFERENCE, μM/ml.
1.0
0.8
0.6
0.4
0.2
0
CONTROLS
ACROMEGALY
CUSHINGS
STARVATION
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 3.

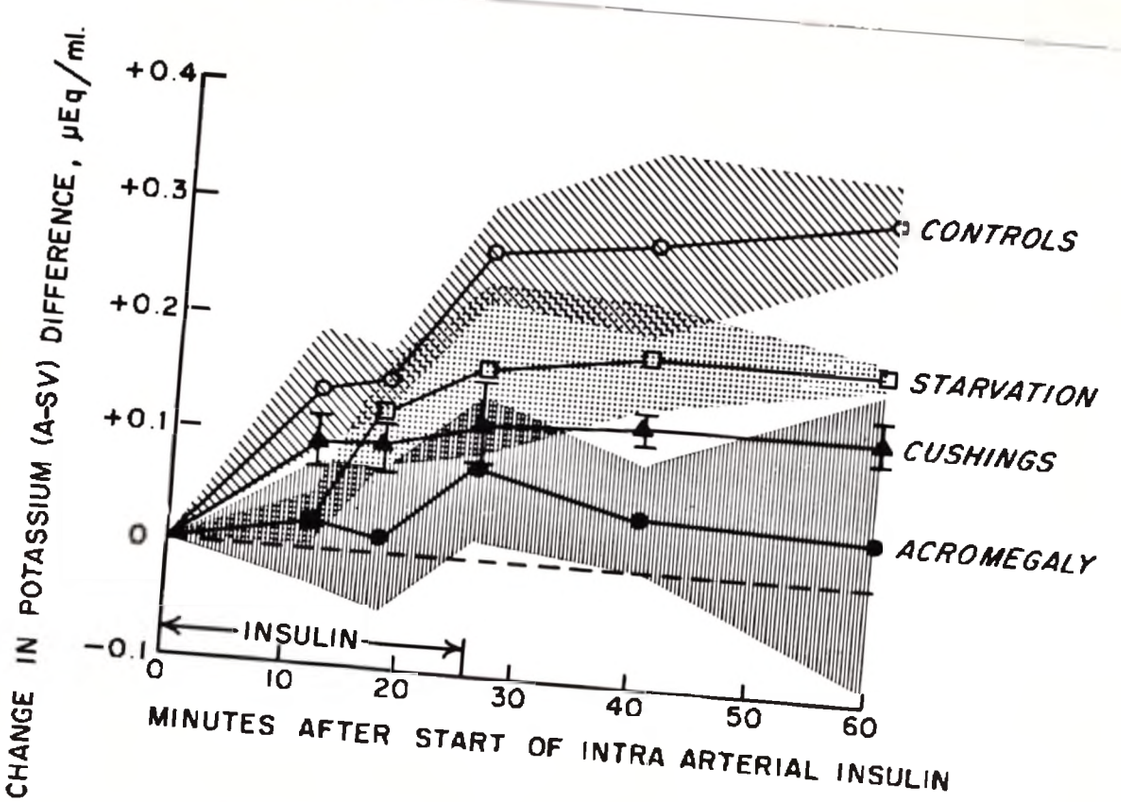

CHANGE IN POTASSIUM (A-SV) DIFFERENCE, μEq/ml.
+0.4
+0.3
+0.2
+0.1
0
−0.1
CONTROLS
STARVATION
CUSHINGS
ACROMEGALY
INSULIN
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA ARTERIAL INSULIN

FIG. 4.

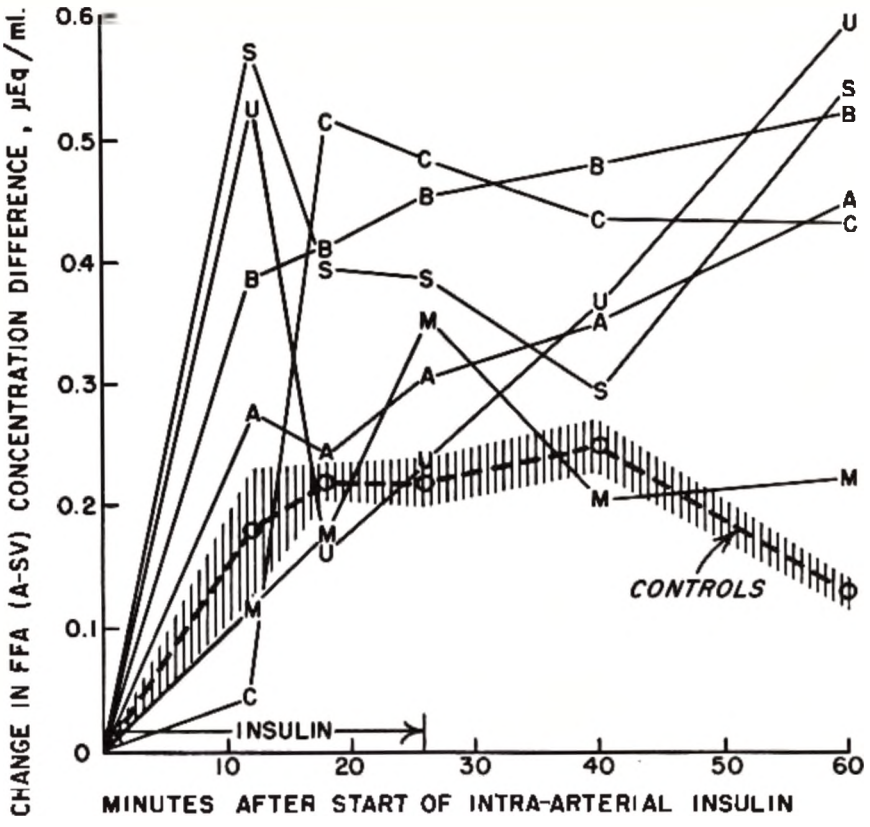

CHANGE IN FFA (A-SV) CONCENTRATION DIFFERENCE, μEq/ml.
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN
INSULIN
CONTROLS

FIG. 5.

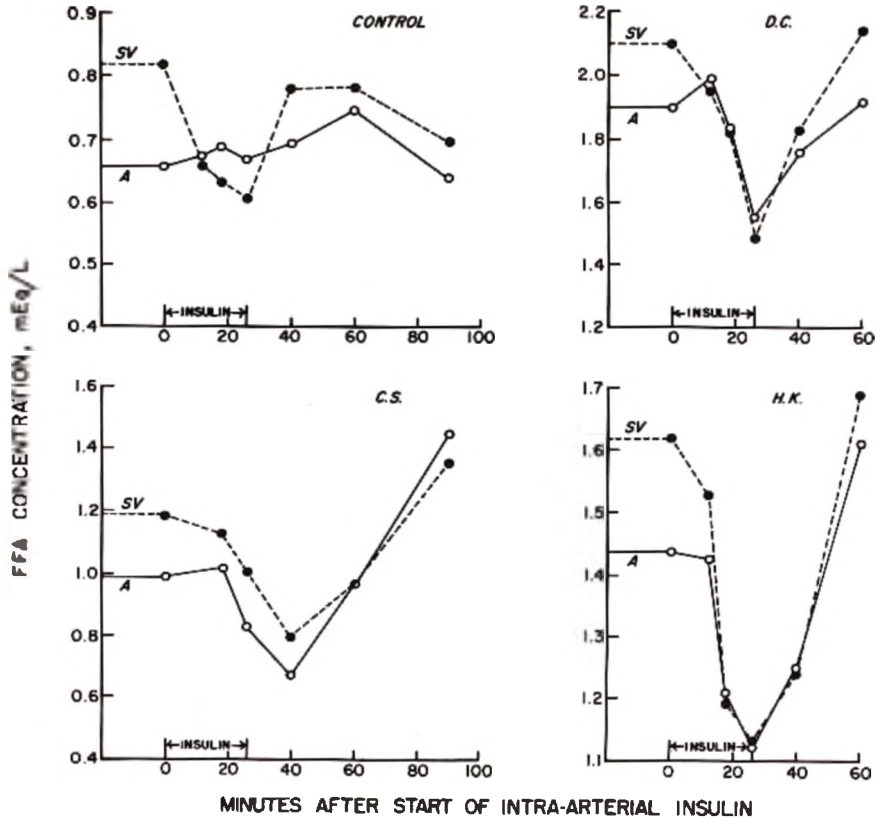

CONTROL
D.C.
C.S.
H.K.
SV
A
INSULIN
FFA CONCENTRATION, mEq/L
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 6.

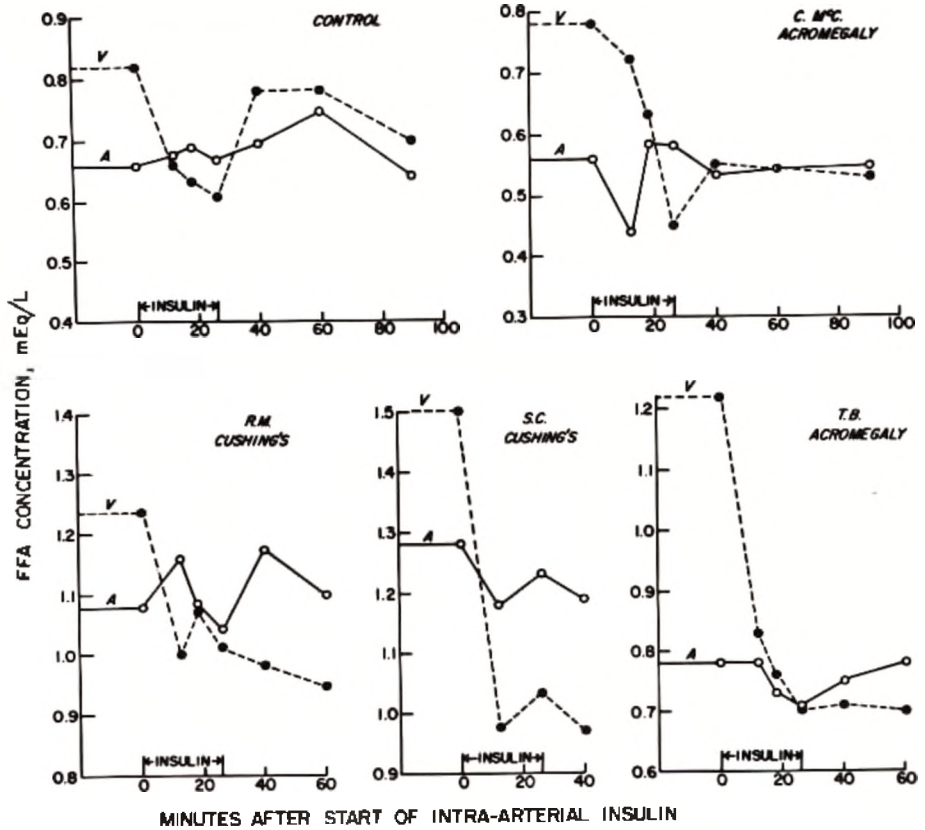

CONTROL
C. M°C. ACROMEGALY
R.M. CUSHING'S
S.C. CUSHING'S
T.B. ACROMEGALY
V
A
INSULIN
FFA CONCENTRATION, mEq/L
MINUTES AFTER START OF INTRA-ARTERIAL INSULIN

FIG. 7.

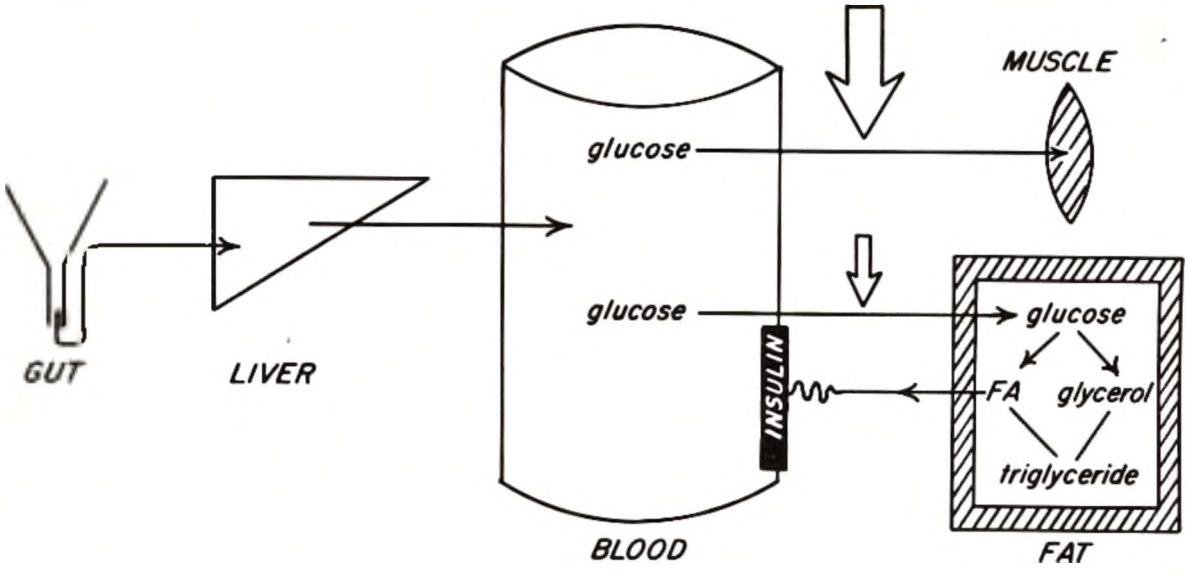

MUSCLE
glucose
glucose
glucose
INSULIN
FA
glycerol
triglyceride
GUT
LIVER
BLOOD
FAT

FIG. 8.

CHAPTER VIII

EFFECTS OF HUMAN GROWTH HORMONE ON FOREARM METABOLISM IN MAN

It has been recognized for many years that the pituitary gland elaborates either one, or a family of hormones which are essential for normal growth. During the past decade, a number of investigators have prepared protein extracts from human pituitary glands which fulfill some of the criteria for the previously elusive hormone, human growth hormone (H.G.H.) (1, 2, 3). The HGH content of the pituitary does not vary with age (4) and assays of plasma HGH activity have revealed that levels in children and adults do not differ (5). This feature has been consistently observed, although the absolute levels of plasma HGH when measured by red cell agglutination techniques (5, 6, 7) have been about ten times greater than that reported recently by Daughaday and colleagues, using a radioimmuno-assay procedure (8).

Considerable interest has been aroused, in consequence, as to the function of HGH in body economy in adult life, that is, after cessation of linear growth. Investigation of this problem has usually entailed injection, either by intramuscular or intravenous route, of fairly large doses of HGH, and subsequent observation of the effects on systemic levels, and total body balance, of selected metabolites. Several inconsistencies have been apparent in the reported observations, particularly with respect to carbohydrate metabolism, both hypo- and hyper- glycemia being recorded (9, 10, 11, 12), and with respect to fat metabolism, controversy being centered on whether or not the HGH induced hyperlipacidemia involves a direct lipolytic action of HGH on adipose tissue (12, 13, 14).

We have previously described a technique for appraisal of metabolism of forearm tissues, chiefly skeletal muscle and adipose tissue, in situ (15). This technique lends itself nicely to examination of local effects of hormones, which can be injected into the brachial artery, along with blue dye, in amounts large enough to affect local metabolism, without inducing, in general, any systemic response. The effects of the hormone are therefore as far as possible not complicated by any counter-regulatory mechanisms the body may institute. This is particularly important with respect to HGH which has been shown to provoke, either directly or indirectly, increased insulin secretion by the pancreas (16).

Effects on forearm metabolism of HGH, administered in microgram amounts by close intra-arterial injection, were examined in five young subjects. Answers were sought to the following questions (1) Does HGH influence the uptake of glucose by forearm tissues? (2) Is lactate production by forearm tissues affected, either directly, or secondary to an effect on glucose uptake? (3) Does HGH influence K^+ movement across forearm muscle? In particular, does it reverse the characteristic net movement of K^+ out of forearm muscle noted in control subjects, under the conditions of our experiments (15). (4) In the light of evidence demonstrating significant increase in O_2 consumption by forearm muscle in acromegaly (17), can acute administration of HGH reproduce this effect? and (5) What effects are seen following HGH

administration on FFA movement in the forearm, with respect to both output by adipose tissue, and uptake by skeletal muscle?

METHODS

Five young male subjects of normal body weight were studied. Studies were performed during the morning hours 8 A.M. to 12 noon, that is, 12-16 hours after the last meal. Previous intake of carbohydrate was normal. The brachial artery (A), a draining forearm deep vein (DV) and a draining forearm superficial vein (SV) were catheterised by techniques described previously (15). Blood flow to the forearm was measured by indicator-dilution techniques based on continuous infusion of the dye, T-1824, into the brachial artery. Uptake (or production) of a metabolite by forearm muscle calculated from the equation $\dot{Q} = F(A - DV)$ where $\dot{Q}$ = uptake (or production) by forearm muscle of the metabolite in units of mass per minute per 100 ml forearm, F = blood (or plasma) flow in units of ml per minute and A and DV are arterial and deep venous blood (or plasma) concentrations in units of mass per ml. A - SV concentration differences reflect metabolism of skin and subcutaneous adipose tissue in the main. The errors and assumptions have been discussed elsewhere (18).

At least two sets of samples were taken under basal postabsorptive conditions. Basal values are the means of these estimations. HGH (let NIH-GH HS 44 B) was then infused by constant intra-

arterial injection in a dose of 0.2 μgm per kg per min. for 26 minutes. Samples were taken 12, 18 and 26 minutes following the start of the HGH infusion and for periods up to one hour thereafter. Circulation to the hand and wrist was excluded during, and for five minutes prior to withdrawal of blood samples, and for the entire period of HGH administration.

RESULTS

Results appear in Table I.

Arterial concentration of HGH: The calculated concentration of HGH in brachial arterial plasma ranged from 300 to 700 millimicrons per ml plasma. At the time these studies were performed, we were under the impression that the concentration of HGH we were administering was simply doubling the endogenous concentration of HGH (5). From the subsequent studies of Daughaday et al (8) it is probable that the levels approached ten times the normal concentration of HGH in the basal state.

Blood flow: Blood flow remained acceptably constant in these experiments, that is, it did not vary by more than 20 per cent about the mean in every case.

Arterial concentration: Mean changes in arterial glucose, lactate and potassium concentrations were in a direction expected from the observed local response, but were small, less than 5 per cent, in each case. Changes in arterial FFA concentrations were larger; significant arterial hyperlipacidemia was recorded in four of the five subjects.

Glucose: A prompt decrease in glucose A - DV concentration differences and glucose uptake (Q_G) of forearm muscle occurred following HGH administration. At peak effect, glucose uptake had dropped to about one quarter of the basal value, and the decrease persisted for the entire period during which observations were made. (Fig. 1).

Lactate: Lactate production fell significantly following HGH administration, presumably secondary to the decreased uptake of glucose by forearm muscle (Fig. 2). Since glucose uptake fell to a greater extent than did lactate production, the L/G ratio, which defines the fraction of glucose uptake accounted for by lactate production, rose from 0.4 basally, to 0.8 during HGH administration.

O_2, CO_2, and R.Q: No changes were observed in O_2 consumption following HGH administration. The striking increase in forearm muscle Q_{O_2} noted in acromegaly (17) was not reproduced by acute administration of HGH. A small, but significant fall in R.Q. was, however, consistently recorded.

Potassium: HGH reversed the characteristic net movement of potassium out of forearm muscle into venous plasma (15). This effect, clearly visible in 4 of the 5 subjects when measured at 12 minutes, reached its maximum at 18 minutes, and then plateaued for the entire period of observation (Fig. 3).

Glucose, lactate and potassium A - SV concentration differences:

Changes in A - SV glucose, lactate and potassium A - SV differences parallelled those observed in A - DV concentration differences.

FFA: Arterio-superficial FFA concentration differences, which reflect discharge of FFA from subcutaneous adipose tissue into venous plasma, clearly increased (i.e., became more negative) following HGH (Fig. 4). This is consistent with the notion that HGH promotes release of FFA from adipose tissue. In contrast to the prompt changes in glucose and potassium, this effect is delayed, becoming significant 40 minutes after the start of the HGH infusion. This suggests that this action may well be indirect. The arterial hyperlipacidemia noted in four of the five subjects is consistent with this view, and suggests that this may be the most sensitive index of HGH activity. A systemic effect was recorded in response to an estimated HGH concentration of 44 mμgm per ml recirculating plasma.

Owing to anatomical considerations, the Fick principle is not entirely applicable to assessment of FFA metabolism by human forearm muscles. Deep venous effluent drains not only muscle but also adipose tissue which is streaked intimately among muscle fibres. A - DV concentration differences, therefore, reflect simultaneous uptake of FFA by skeletal

muscle and release by adipose tissue, which complicates analysis of effects of HGH. A - DV FFA concentration differences became positive or more positive following HGH administration (Fig. 5). In the light of the ability of HGH to increase FFA release from the superficial bed, it may have been anticipated that FFA A - DV differences, which of course drains an uncertain amount of intermuscular adipose tissue, would be reduced or even become negative. The percentage of forearm muscle O_2 uptake accounted for by FFA oxidation, FFA/O_2, actually increases from an apparent basal value of 0.15 to 0.72, under maximal HGH stimulation. This favors the view that HGH increases the rate of oxidation of mobilised fatty acids.

DISCUSSION

The human forearm is sensitive to HGH in concentrations of several hundred millimicrograms per ml. A prompt effect was noted on glucose uptake by forearm muscle which was reduced to 25 per cent of its basal value. Demonstration of a direct inhibitory effect of HGH on glucose uptake by forearm muscle is in keeping with the so-called 'diabetogenic' properties of the hormone demonstrated by earlier workers (19, 20).

If the response of forearm skeletal muscle is typical for all body skeletal muscle, then the frequently recorded hyperglycemic properties of HGH become readily explicable. A small rise

in arterial glucose was actually observed by us. Our observations do not, however, resolve the well documented "early hypoglycemic" effects of HGH which follow the systemic administration of large doses of HGH (9, 10). Presumably this is an indirect effect, in part perhaps a consequence of enhanced insulin release from the pancreas (16).

Of considerable interest was the observation that lactate production decreased as glucose uptake decreased. This tends to support the view that lactate production by forearm muscle is influenced by the magnitude of glucose uptake, even in the face of vigorous O_2 consumption by forearm muscle (15, 18). Concomitant with the decrease in glucose uptake, there was a fall in the G-L/O_2 ratio, that is, the fraction of O_2 consumption accounted for by glucose oxidation, from 0.27 to 0.04. The effects of HGH on FFA metabolism are probably mediated by both an increase in uptake by forearm muscle and an increased release from adipose tissue. Because of the exquisite sensitivity of arterial FFA levels following HGH, we have been unable to establish conclusively whether the lipolytic effects are direct or indirect. However, the marked delay before the effect becomes apparent favors the latter. This is in keeping with evidence, based on studies with excised rat adipose tissue, that the in vitro lipolytic properties of growth hormone preparations are either inconstant, or only achieved when exceedingly high concentrations are employed (13). The observed increase in positive A - DV FFA differences and

FFA/O_2 ratios, favors the view that HGH increases FFA uptake by skeletal muscle. The notion that growth hormone increases fatty acid oxidation was advanced by Greenbaum and McLean (21) working with rat liver slices, and is supported by the elegant in vivo isotopic studies of de Bodo and colleagues in dogs (22). Examination of the R.Q. of forearm muscle, which falls following HGH administration, suggests that combustion of fatty acids is proceeding to 2-Carbon fragments more rapidly than can be accomodated by the tricarboxylic acid cycle. Unfortunately, our estimations of R.Q. are too infrequent and fragmentary to establish either the temporal duration of this fall, or whether there was any subsequent rise of R.Q. which would attend the eventual complete combustion of 2-Carbon fragments.

SUMMARY

Forearm tissues are extremely sensitive to administration of HGH in a concentration of several hundred m/gm per ml brachial arterial plasma. HGH promptly promotes entry of potassium into forearm muscle, and reduces glucose uptake to one-fourth basal values, with a concomitant decrease in lactate production by the forearm. The effects of HGH on FFA metabolism are probably mediated by both an increase in FFA uptake by forearm muscle, and an increased release of FFA from forearm adipose tissue. The latter effect is probably indirect, and is accompanied by significant hyperlipacidemia. This probably constitutes the most sensitive index of HGH activity.

REFERENCES

1. Wilhelmi, A.E. Systematic fractionation of human pituitaries. Ciba Foundation Colloquia on Endocrinology. Wolstenholme, G.E.W., and O'Connor, C.M., Eds. Vol. XIII, Little Brown and Company, 1959, p. 25.
2. Raben, M.S. Preparation of growth hormone from pituitaries of man and monkey. Science, 1957, 125, 883.
3. Li, C.H., and Papkoff, H. Preparation and properties of growth hormone from human and monkey pituitary glands. Science, 1956, 124, 1293.
4. Gershberg, H. Growth hormone content and metabolic actions of human pituitary glands. Endocrinology, 1957, 61, 160.
5. Read, C.H., and Bryan, G.T. The immunological assay of human growth hormone. Recent Progr. in Hormone Res. 1960, 16, 187.
6. Hartog, M., and Fraser, R. The immunological assay of growth hormone in human serum. J. Endocrinol. 1961, 22, 101.
7. Ehrlich, R.M., and Randle, P.J. Immunoassay of growth hormone in human serum. Lancet, 1961, ii. 230.
8. Utiger, R.D., Parker, M.L., and Daughaday, W.H. Studies on human growth hormone. I. A radio-immunoassay for human growth hormone. J. clin. Invest. 1962, 41, 254.
9. Pearson, O.H., Dominguez, J.M., Greenberg, E.J., Pazianos, A., and Ray, B.S. Diabetogenic and hypoglycemic effects of human growth hormone. Tr. A. Am. Physicians, 1960, 73, 217.

10. Zahnd, G.R., Steinke, J., and Renold, A.E. Early metabolic effects of human growth hormone. Proc. Soc. Exper. Biol. and Med. 1960, 105, 455.

11. Committee on Medical Research Council. Effectiveness in man of human growth hormone. Lancet, 1959, 1, 7.

12. Raben, M.S. Growth Hormone I Physiological Aspects. New Eng. J. Med. 1962, 266, 31.

13. White, J.E., and Engel, F.L. Lipolytic action of corticotropin on rat adipose tissue in vitro. J. clin. Invest. 1958, 37, 1556.

14. Krahl, M.E. Effect of pituitary hormones on metabolism of isolated tissues in International Symposium on The Hypophyseal Growth Hormone, Nature and Actions, Smith, R.W., Jr., Gaebler, O.H., and Long, C.N.H., Eds. New York, Blakiston Division of McGraw-Hill Book Company, 1955, p. 369.

15. Baltzan, M.A., Andres, R., Cader, G., and Zierler, K.L. Heterogeneity of forearm metabolism with special reference to free fatty acids. J. clin. Invest. 1962, 41, 116.

16. Randle, P.J. in Diabetes, Williams, R.H., Ed. New York, Hoeber, 1960, p. 257.

17. Rabinowitz, D., and Zierler, K.L. Forearm metabolism in acromegaly. Program of forty-fourth meeting, The Endocrine Society, abstract 63, p. 31, Chicago, June 1962.

18. Andres, R., Cader, G., and Zierler, K.L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen

and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, 35, 671.

19. Young, F.G. Permanent experimental diabetes produced by pituitary (anterior lobe) injections. Lancet, 1937, 2, 372.

20. Houssey, B.A., and Rodriguez, R.R. Diabetogenic action of different preparations of growth hormone. Endocrinology, 1953, 53, 114.

21. Greenbaum, A.L. Growth hormone and fat metabolism, in The Hypophyseal Growth Hormone, Nature and Actions. Smith, R.W., Jr., Gaebler, O.H., and Long, C.N.H., Eds. Mc Graw-Hill, 1955, p.330.

22. Winkler, B., Steele, R., Altszuler, N., Dunn, A., and deBodo, R.C. Effects of growth hormone on FFA metabolism. Fed. Proc. 1962, 21, 198.

Table I

Mean Effect of H.G.H. on Forearm Metabolism

Time after H.G.H. started	Plasma flow ml/min/ 100 ml arm	A-DV Glucose μM/ml	$\dot{Q}$ Glucose	A-DV Lactate μM/ml	$\dot{Q}$ Lactate	A-DV Potassium μM/ml	$\dot{Q}$ Potassium	R.Q.
0	2.3 ± 0.19	0.24 ± 0.052	0.85	-0.18 ± 0.057	0.74	-0.23 ± 0.077	-0.59	0.75 ± 0.049
12	2.3 ± 0.25	0.08 ± 0.038	0.38	-0.15 ± 0.038	0.57	-0.03 ± 0.046	-0.07	
18	2.3 ± 0.28	0.15 ± 0.027	0.58	-0.09 ± 0.018	0.35	+0.09 ± 0.035	+0.22	
26	2.4 ± 0.19	0.06 ± 0.043	0.18	-0.08 ± 0.028	0.32	+0.10 ± 0.051	+0.25	0.61 ± 0.036
40	2.2 ± 0.30	0.16 ± 0.036	0.58	-0.09 ± 0.033	0.30	+0.12 ± 0.032	+0.31	
60	2.3 ± 0.23	0.12 ± 0.025	0.42	-0.15 ± 0.033	0.49	+0.13 ± 0.025	+0.29	0.63 ± 0.015

Values for A-DV differences are expressumed as μM/ml and values for $\dot{Q}$ are μM/min/100 ml arm. All values are means ±S.E. of means. S.E. of means for $\dot{Q}_{glucose}$, $\dot{Q}_{lactate}$ and $\dot{Q}_{potassium}$ appear in Figures I, II and III. H.G.H. administered for 26 minutes beginning at time zero.

LEGEND TO FIGURES

Fig 1. Glucose uptake by forearm tissues in response to intra-arterial H.G.H. Data are plotted as means ± S.E. of means.

Fig. 2. Lactate production by forearm tissues in response to intra-arterial H.G.H. Data are plotted as means ± S.E. of means.

Fig. 3. Effect of H.G.H. on forearm potassium uptake, expressed as the increase in net inward movement of potassium, compared to potassium movement at time zero. Data are plotted as means ± S.E. of means.

Fig. 4. Effects of H.G.H. on A - SV FFA differences in the human forearm. Data are plotted as means ± S.E. of means.

Fig. 5. Effect of H.G.H. on A - DV FFA differences in the human forearm. Data are plotted as means ± S.E. of means.

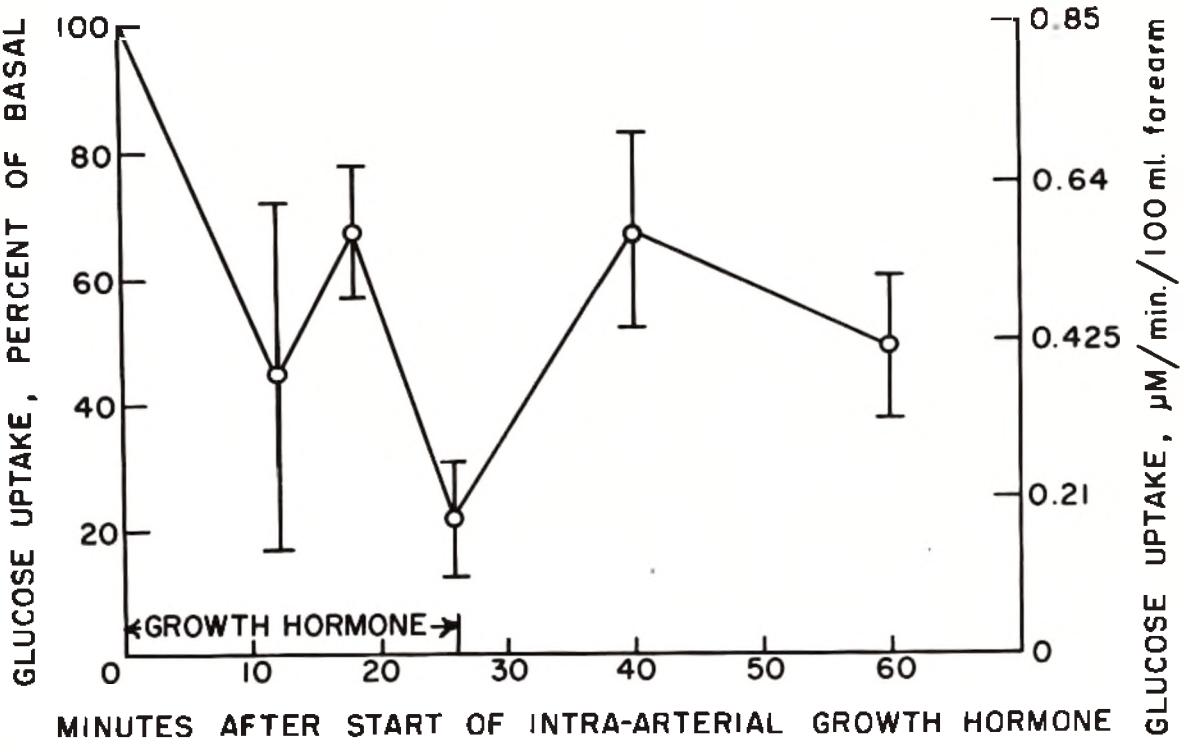

GLUCOSE UPTAKE, PERCENT OF BASAL
100
80
60
40
20
0
0.85
0.64
0.425
0.21
0
GLUCOSE UPTAKE, μM/min./100 ml. forearm
GROWTH HORMONE
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL GROWTH HORMONE

FIG. 1

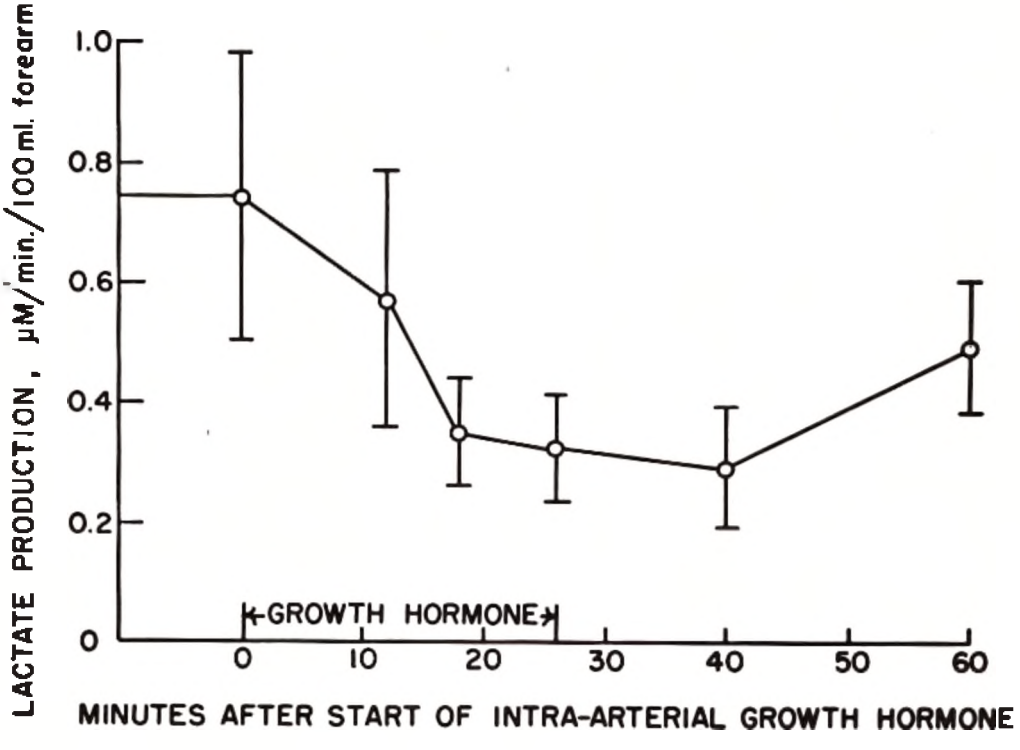

LACTATE PRODUCTION, μM/min./100 ml. forearm
1.0
0.8
0.6
0.4
0.2
0
GROWTH HORMONE
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL GROWTH HORMONE

FIG. 2.

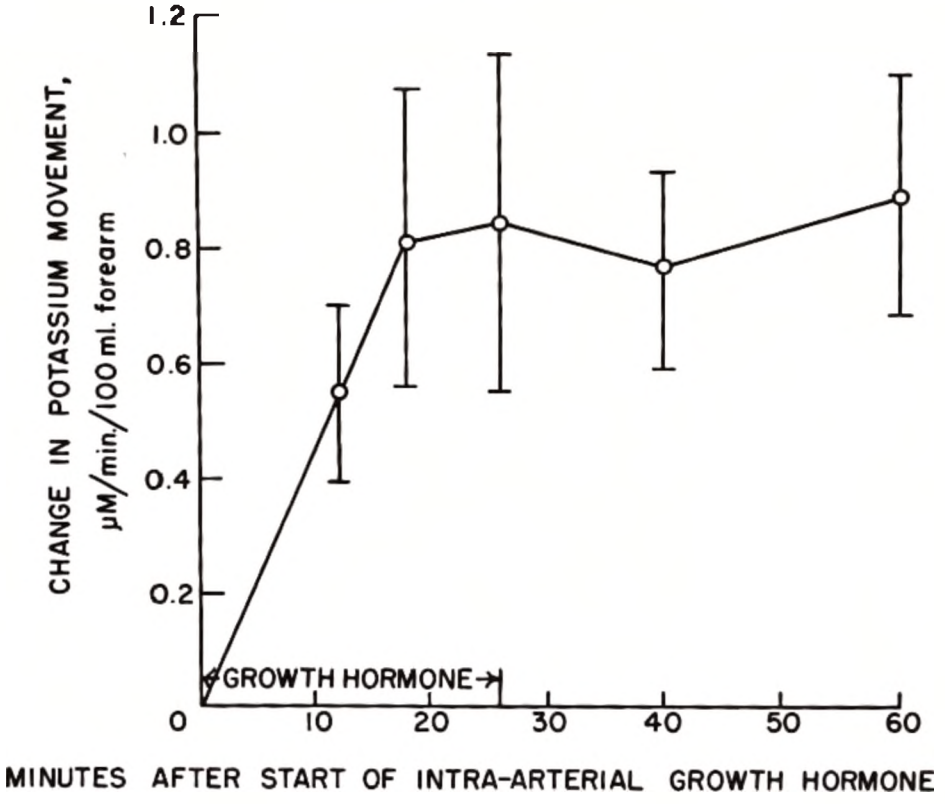

CHANGE IN POTASSIUM MOVEMENT, μM/min./100 ml. forearm
1.2
1.0
0.8
0.6
0.4
0.2
0
GROWTH HORMONE
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL GROWTH HORMONE

FIG 3

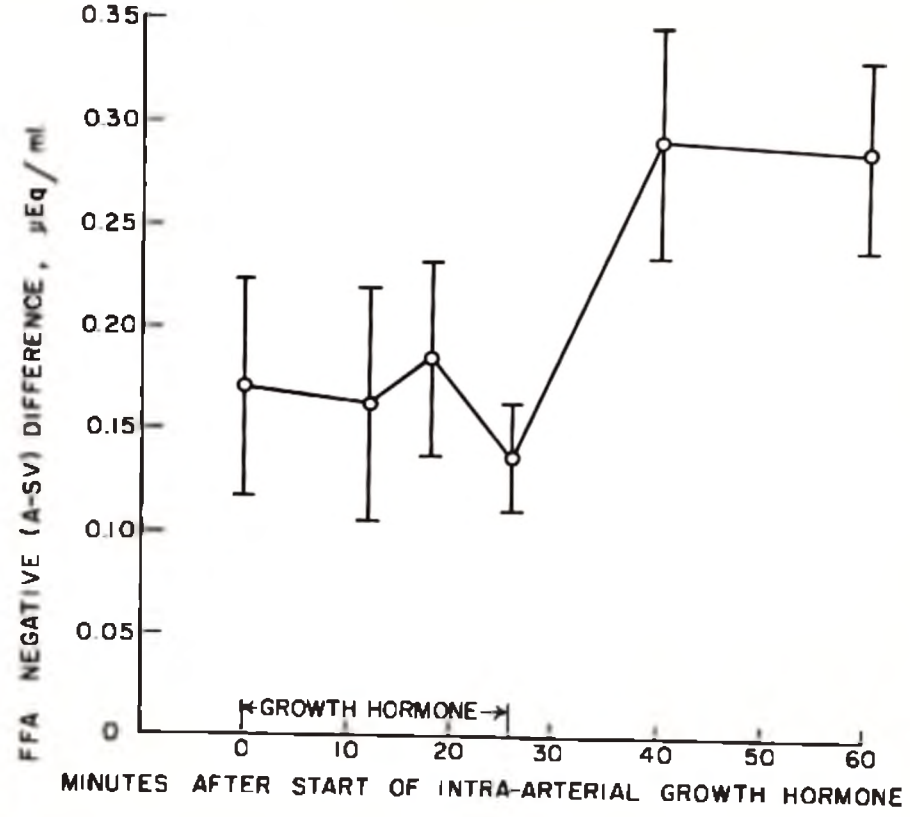

FFA NEGATIVE (A-SV) DIFFERENCE, μEq / ml
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
GROWTH HORMONE
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL GROWTH HORMONE

FIG. 4.

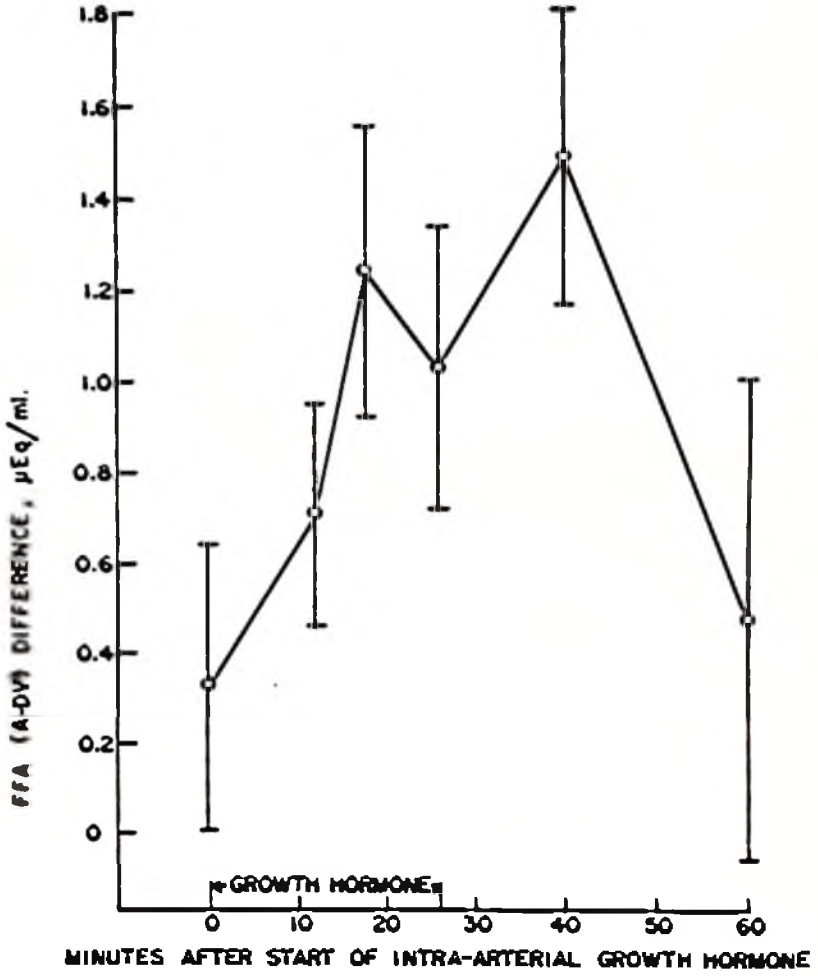

1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
FFA (A-DV) DIFFERENCE, μEq/ml.
GROWTH HORMONE
0
10
20
30
40
50
60
MINUTES AFTER START OF INTRA-ARTERIAL GROWTH HORMONE

FIG. 5.