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*ON THE ACTION OF HORMONES WHICH ACCELERATE THE RATE OF OXYGEN CONSUMPTION AND FATTY ACID RELEASE IN RAT ADIPOSE TISSUE IN VITRO**

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It has become apparent from recent studies in this laboratory and those of others that certain hormones have the common property of stimulating three different metabolic processes in rat adipose tissue *in vitro*. Adrenaline, ACTH, glucagon, and TSH all accelerate in this tissue the rate of oxygen consumption, glycogen breakdown, and fatty acid release (Table 1). We wish to present here data which lend additional support to these observations and which permit the addition to this list of the fat mobilizing substance (F. M. S.) reported by Chalmers *et al.*[14] Data are also presented on the ability of the two peptides prepared from pituitary by Astwood *et al.*[30] to stimulate oxygen uptake *in vitro* by adipose tissue from the rabbit but not from the rat.

TABLE 1

A LIST OF HORMONES WHICH ACCELERATE OXYGEN UPTAKE, FATTY ACID RELEASE, AND GLYCOGEN BREAKDOWN IN ADIPOSE TISSUE WITH PERTINENT REFERENCES

Hormone	Oxygen uptake	Fatty acid release	Glycogen breakdown
ACTH	2, 3, 4	13, 18, 19, 20	10, 11, 21
Adrenaline	1, 2, 3	1, 9, 15, 16	10, 11, 21
Glucagon	2, 5	5, 12	5, 10, 11, 21
F.M.S.	7	7, 14	10
TSH	6, 7	4, 6	10
Growth hormone preparation*	2, 8	17, 18	10

* Recent work[4] indicates that the action of the growth hormone preparation is most likely due to its content of TSH.

As described by Chalmers *et al.*,[14] F.M.S. is excreted in the urine of humans who are fasting and actively mobilizing and utilizing fat. It is polypeptide-like in nature, causes transient hypoglycemia, ketonaemia, and increased mobilization and catabolism of fat upon injection into mice. It is an effective stimulator of the release of free fatty acids from rat adipose tissue *in vitro*. Though the pituitary appears to be necessary for its production, F.M.S. is apparently neither growth hormone nor corticotropin. The pituitary peptides of Astwood *et al.*[30, 31] stimulate

the release of fatty acids from the adipose tissue of rabbits but are much less effective in this regard on the adipose tissue of rats.

An explanation for the dual stimulatory action of all these hormones on oxygen consumption and fatty acid release in adipose tissue is offered. It is proposed that the stimulation of oxygen uptake is secondary to the enhanced liberation of fatty acids by these hormones and that it is a reflection of an increased consumption of high energy phosphate in a process whereby re-esterification of the fatty acids occurs.

Methods.—Male albino rats purchased from the Holtzman Company and weighing 140–180 gm were employed. The precautions observed in the care and handling of animals as well as the procedure for removal of the epididymal fat body have been described previously.[22, 24] Oxygen consumption was determined manometrically at 37° with air as the gas phase. The basic incubation medium was 3.0 ml of Krebs-Ringer phosphate medium, pH 7.4[23] modified to contain one-half the recommended calcium. When glucose was present its concentration was 3 mg per ml. In some experiments human serum albumin (fraction VR, Protein Foundation) was dissolved in the medium to yield a 5% solution and the pH restored to 7.4 by the addition of 0.15 ml of 1 *N* NaOH per 10 ml. The center well of the flasks (15–17 ml total capacity) contained 0.2 ml of 20% KOH and a roll of filter paper. Net gas exchange was determined as described previously.[24] Free fatty acid release was measured by the method of Dole[25] with slight modification, glucose by the method of Somogyi-Nelson,[26, 27] and lactate by the method of Barker and Summerson.[28]

The sample of F.M.S. employed was kindly donated by T. M. Chalmers. The insulin was a crystalline preparation (Lot #466368) supplied through the courtesy of Eli Lilly and Co. E. B. Astwood generously furnished samples of pituitary fractions I, and I and II, as well as α-MSH. The sample of TSH assayed six units per mg and was the gift of A. E. Wilhelmi.

Results.—The effect of the addition of F.M.S. upon the oxygen consumption of adipose tissue is illustrated by the data presented in Figure 1. Here two different experiments are shown, in each, paired tissues from a single rat were employed. In one experiment, portrayed in the left half of Figure 1, one piece of tissue was placed into medium that contained 0.1 unit (4 μg) of insulin per ml and the other piece into medium without insulin. Glucose was present in both flasks. The rate of O_2 consumption per 100 mg wet weight of tissue during an initial 30-minute period was determined, and then F.M.S. was added from the side arm of the flask to yield a final concentration of 33 μg per ml. In both flasks a prompt stimulation of oxygen uptake occurred which was much more marked in the presence of insulin than in its absence. An inhibition of O_2 uptake soon set in when insulin was absent so that by the third hour of the experiment the rate had fallen to about 50 per cent of the initial value. Previous experiments from this laboratory have shown that the rate of O_2 consumption of untreated tissues remains nearly linear over a three-hour period and that under conditions similar to those used here the addition of insulin alone has little effect upon the O_2 consumption.[1, 8]

In the experiment shown in the right half of Figure 1, one piece of tissue was placed into medium containing glucose and 5 per cent albumin while the paired piece of tissue was incubated in medium containing glucose and no albumin. Again after an initial 30-minute period F.M.S. was added to both flasks to yield a final concentration of 33 μg per ml. In the presence of albumin the stimulation of O_2 uptake that results initially is maintained. In contrast, a progressive inhibition in rate is observed with the paired tissue. These results are similar to those seen when the same type of experiments are performed with adrenaline.[1]

In Table 2 are given the results of experiments in which the concentration of

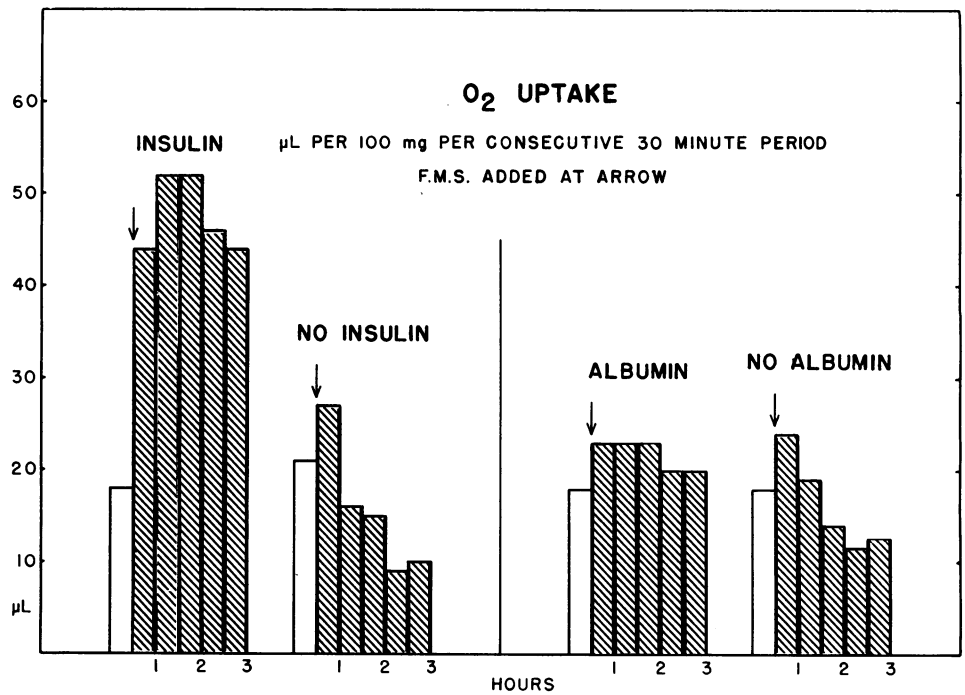

FIG. 1.—The effect of fat mobilizing substance (F.M.S.) on the oxygen consumption of rat adipose tissue. Experimental conditions as described in the text. The concentration of F.M.S. was 33 μg per ml, and when present, insulin 0.1 unit (4 μg) per ml, albumin 50 mg per ml.

F.M.S. was varied. Each experiment was performed in a manner similar to the ones shown in Figure 1, with paired tissues from a single rat being employed in each case. Unless otherwise stated, glucose (3 mg/ml) and insulin (0.1 u/ml) were present from the start. Under these conditions the increase in rate of O_2 consumption produced is a function of F.M.S. concentration within the range 1.0 to 33 μg/ml. Concentrations higher than 33 μg/ml were not tried since the supply of F.M.S. was limited. Previous experience with other hormones has shown that the limit of the tissue's ability to consume O_2 is reached in the vicinity of a 200 per cent in-

TABLE 2

THE EFFECT OF ALTERATIONS IN F.M.S. CONCENTRATION AND MEDIUM COMPOSITION ON THE OXYGEN CONSUMPTION OF RAT ADIPOSE TISSUE

Experiment	F.M.S., μg/ml	O_2 Uptake,* μl per hr per 100 mg Initial	After F.M.S.	% Change	Comment
1 A	33.3	35	105	+200	
B	3.3	34	67	+97	
2 A	3.3	46	90	+96	
B	3 3	36	42	+17	No glucose
3 A	1.0	26	30	+15	
B	1.0	28	31	+11	No insulin
4 A	33.3	34	104	+206	
B	33.3	40	20	−50	No insulin
5 A	10.0	30	18	−40	No insulin
B	10.0	33	44	+33	5% albumin and no insulin

* The initial rate is determined from a 30-minute period before the addition of F.M.S. while the rate after F.M.S. represents the value for the third hour of the incubation.

crease in rate. The data in Table 2 also show that the presence of glucose is necessary for the effect of insulin to be seen.

In experiment 5 of Table 2, the free fatty acids of the medium and tissue at the end of the 3-hour incubation were determined. In 5*A* the 3.0 ml of medium contained a total of 0.39 μmol of fatty acid per 100 mg of tissue, while each 100 mg of the tissue itself contained 1.71 μmol. When albumin was present (5*B*) the medium gained a total of 2.44 μmol of fatty acid per 100 mg of tissue and the tissue itself contained 1.19 μmol per 100 mg.

TABLE 3

THE EFFECT OF F.M.S. ON THE METABOLISM OF RAT ADIPOSE TISSUE IN A BICARBONATE BUFFERED MEDIUM

	(Experiment 1)		(Experiment 2)	
	Control	F.M.S.	Control	F.M.S.
Net gas exchange	−1.2	−0.4	−2.0	−0.3
Glucose uptake	0.81	1.25	0.62	1.54
Lactate production	0.32	0.50	0.28	0.51
Free fatty acid changes	−0.36	1.93	−0.30	1.41

F.M.S. final concentration 10 μg/ml. All values in μmol per 100 mg of tissue wet weight per 3-hour incubation period. Each flask contained 1.5 ml total fluid volume. Paired tissue from a single rat was employed in each experiment. The medium was Krebs-Ringer bicarbonate modified to contain one-half the recommended calcium. Albumin (5%) and glucose (1.5 mg per ml) were present in all cases. The albumin employed contained a small amount of fatty acid which resulted in the medium's showing an initial value of 0.9 μmol of fatty acid per ml.

The effect of F.M.S. on the metabolism of adipose tissue in a bicarbonate buffered medium containing albumin is shown in Table 3. The amount of fatty acid released into this medium at this concentration of F.M.S. (10 μg/ml) is in reasonable agreement with that reported by Chalmers *et al.*[14] There is a slightly greater uptake of glucose and output of lactic acid by the tissue in the presence of F.M.S. The net gas exchange of the tissue is also less negative with F.M.S. present. This

TABLE 4

A COMPARISON OF THE EFFECTS OF CERTAIN ANTERIOR PITUITARY FRACTIONS ON THE OXYGEN CONSUMPTION OF RAT AND RABBIT ADIPOSE TISSUE

Substance	Concentration (μg/ml)	Per Cent Increase in Oxygen Consumption Rat	Rabbit
Peptide I	5	0 (1)	98 (1)
Peptide I & II	5	0 (6)	87 (5)
TSH	5	163 (6)	10 (5)
	1	156 (12)	
	0.1	50 (4)	
α-MSH	5.0	39 (1)	134 (1)

Values are the average of the number of experiments indicated by the figures in parentheses. Experiments were run in the manner previously described with insulin (0.1 unit/ml) and glucose present (3 mg/ml). In some experiments albumin was also present (5%) but it was without effect upon the response obtained. Epididymal fat body was the source of tissue in the rat experiments while in the rabbit perirenal and omental adipose tissue were employed.

may be a reflection of CO_2 release from the bicarbonate of the medium due to the increased acid production. In other experiments in which the final concentration of F.M.S. was 33 μg per ml the net gas exchange of the tissue also remained negative. Moreover, no effect of F.M.S. at this concentration was found on the net gas exchange response of the tissue to 0.1 unit of insulin per ml. In this respect the action of F.M.S. is unlike that of adrenaline.[29]

Astwood *et al.*[30] have described the preparation from porcine anterior pituitary lobes of two peptides which are active in releasing fatty acids from the perirenal or omental adipose tissue of rabbits. They are relatively inactive in this respect on the epididymal fat pad of rats.[31] In Table 4 data are presented which show that

these fractions are capable of stimulating oxygen consumption of adipose tissue from the rabbit but not from the rat. Also presented in this table are data on the action of TSH. This substance is very active in stimulating the oxygen consumption of rat adipose tissue but is relatively inactive on rabbit adipose tissue. A species difference thus appears to exist in the response of adipose tissue to these pituitary fractions. A similar situation may exist with regard to α-MSH but the limited amount of this material available to us permitted only one experiment to be performed.

Discussion.—It is possible to offer an explanation for the effect of the hormones listed in Table 1 upon the oxygen uptake of adipose tissue which is based upon their ability to increase the production of unesterified fatty acids in this tissue. The schema in Figure 2 is presented to aid in this explanation. The accumulated fat

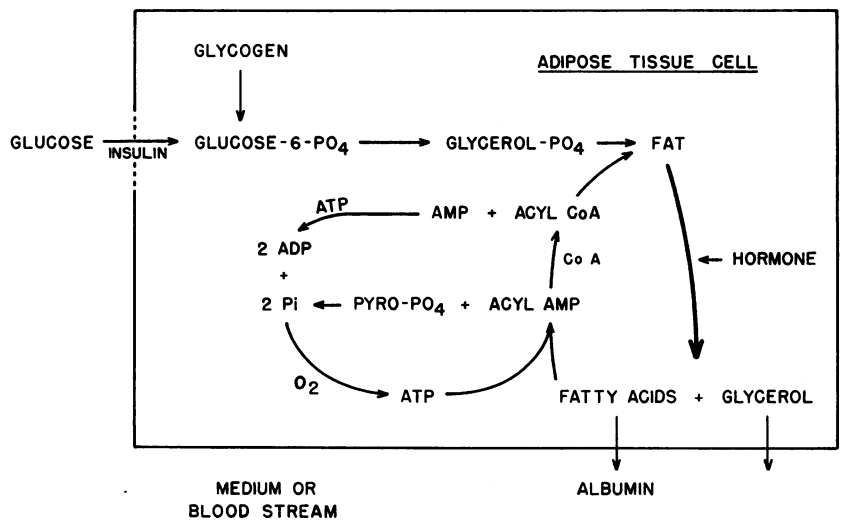

FIG. 2.—A schema offered as an aid in the explanation of the concomitant stimulation of oxygen consumption and fatty acid release in adipose tissue by certain hormones.

within the adipose tissue cell is visualized as being in a dynamic steady state with respect to the pool of fatty acids in the tissue. Thus under normal conditions a continual hydrolysis of fat to fatty acids and glycerol is envisioned which is balanced by a re-esterification of the fatty acids to form new triglycerides. In the presence of the hormones under discussion an acceleration of the lipolysis of fat to fatty acids and glycerol takes place. As fatty acids begin to accumulate in the tissue, two pathways are portrayed as open to them; they may leave the tissue to enter an albumin-fortified medium or the blood stream, or they may undergo re-esterification at an increased rate. In addition, though not indicated in Figure 2, they may also proceed through acyl CoA to steps leading to their oxidation. The proportion of fatty acid following each of these pathways will depend on certain other factors. Now as shown in Figure 2 the re-esterification process requires energy which is furnished by ATP. The resulting breakdown of ATP to ADP and inorganic phosphate (Pi), as indicated, can in turn trigger an increase in oxygen consumption for the regeneration of ATP. The fuel for this oxidative process may be fatty acid, glucose, or both.

The maximum stimulation of oxygen consumption by any of these hormones occurs when both insulin and glucose are present in the incubation medium. The primary reason for this effect is believed to be due to the stimulation by insulin of glucose uptake by the cell and the consequent increased conversion of glucose to glycerol phosphate. Without an adequate supply of this compound re-esterification of fatty acids presumably can not proceed.[32] The glycerol produced during lipolysis apparently cannot be re-utilized to furnish glycerol phosphate due to the absence of glycerol kinase in this tissue.[33] Shapiro *et al.*,[34] Cahill *et al.*,[35] and Lynn *et al.*[3] have shown that radioactive glycerol is not readily converted to either triglyceride glycerol or CO_2 by adipose tissue incubated *in vitro*. It has also been shown that adrenaline[3, 15] and glucagon[5] increase the release of glycerol in addition to free fatty acids from adipose tissue incubated *in vitro*. Hagen[36] has also shown that injection of noradrenaline into rabbits produces a parallel rise in blood free fatty acids and glycerol.

Although all the hormones which cause a release of fatty acids also stimulate oxygen uptake, the patterns of their action are not identical. F.M.S., ACTH, and adrenaline are similar in their action. The addition of any of these compounds may produce a biphasic response in which a stimulation of oxygen uptake occurs first followed by an inhibition. The inhibition sets in more promptly and is more severe when high concentrations of the hormones are employed or when glucose is absent from the medium. The inhibition can be delayed or even prevented by the simultaneous addition of insulin in the presence of glucose. The inhibition may also be diminished or prevented by the inclusion of albumin in the incubation medium. These facts strongly suggest that the inhibition of oxygen uptake is the result of an excessive accumulation of fatty acids within the tissue due either to their failure to leave the tissue or to the inability of the re-esterification process to keep pace with the rate of fatty acid production. Direct analyses of the tissue have shown this to be true.[1, 37] The cause of this inhibition may be related to the fact that fatty acids are inhibitors[38, 39] of the electron transmitter system of the mitochondria. It is also possible that at least part of the initial stimulation of O_2 consumption is due to the ability of fatty acids to uncouple oxidative phosphorylation.[40] The diminution in the supply of ATP that would thus result, would however in time lead to a failure of the re-esterification process with a consequent greater accumulation of fatty acids.

On the other hand with TSH or glucagon an inhibition never occurs and one only sees a stimulation of oxygen consumption. It is believed that this variation in pattern of action of these different hormones *in vitro* is a reflection of their respective potencies as releasers of fatty acids on the one hand and their ability on the other hand to bring about an uptake of glucose by adipose tissue. If the rate of production and accumulation of fatty acids within the tissue exceeds the rate of formation of either glycerol phosphate or of high energy phosphate needed for the re-esterification process then inhibition of O_2 uptake will result.

There is ample evidence in the recent literature to indicate that ACTH and adrenaline release fatty acids either *in vivo* or from adipose tissue incubated *in vitro*. This aspect has been reviewed very adequately (cf. 41 and 42). Also well documented is the fact that the release of fatty acids by these hormones is diminished if both insulin and an ample glucose supply are available.[16, 42] This dim-

inution in release of fatty acids appears not to be due to an inhibition of the conversion of fat to fatty acid but to an acceleration of the process whereby these acids are re-esterified to triglycerides. For example several workers[34, 43, 44] have reported that adipose tissue incubated *in vitro* incorporates labeled free fatty acids from the medium into neutral lipid. Bally *et al.*[43] have shown that the rate of esterification of fatty acids is enhanced by the presence of glucose in the medium and that if insulin is added in addition, a further stimulation in rate may be observed. These workers have suggested that this effect of glucose reflects its need as a source of glycerol phosphate for the esterification process. Evidence that the triglycerides of adipose tissue continuously undergo lipolysis to free fatty acids and the fatty acids are in turn re-esterified has been presented by Cahill *et al.*[45] These workers showed that when adipose tissue is incubated *in vitro* in the presence of radioactively labeled glucose, the glyceride glycerol rapidly became labeled. The amount of this label was out of all proportion to that which would be expected based solely on the *de novo* fatty acid synthesis measured simultaneously. In a later study Cahill *et al.*[46] showed that this incorporation of glucose carbon into glyceride glycerol was enhanced when adrenaline was added and an even further stimulation resulted if insulin was also present. A stimulation of glucose-incorporation into glyceride glycerol was also observed with ACTH and a growth hormone preparation.[18]

Of especial interest are the *in vivo* studies of Davidson *et al.*[47] These workers have reported that the injection of 1 mg of glucagon or 0.1 mg of epinephrine into a rat weighing 200–250 gm produces an increase of about 50 per cent in the rate of O_2 consumption one hour after the injection. This response is markedly reduced in adrenalectomized rats but can be restored by cortisone therapy. This latter point is of particular interest in view of the fact that free fatty acid release from adipose tissue is not induced by epinephrine in adrenalectomized rats[48] or dogs,[49] but can be restored in both cases by cortisone therapy. These findings suggest that the stimulation of oxygen consumption by adipose tissue *in vitro* by these agents may also occur *in vivo* and that a mechanism similar to that discussed here may be functioning. It should be pointed out however that in the intact animal an increase in oxygen consumption may not be solely due to adipose tissue. Fatty acids released into the blood stream by the action of these hormones may undergo re-esterification in other tissues (e.g., liver) with a concomitant increase in O_2 consumption by these tissues.

A process whereby fat is broken down to fatty acid and glycerol with a subsequent re-esterification of the fatty acid by way of glycerol phosphate furnished by glucose seems at first glance to be wasteful and useless. The question may be raised, however, as to whether this process might be utilized by homeothermic animals to generate heat for the maintenance of body temperature. The possible role of brown adipose tissue in this regard would be of especial interest since it is so abundant in hibernating animals and extremely rich in cytochromes.[50] George Cahill has made the interesting suggestion[51] that perhaps subcutaneous fat should be thought of not just as an insulating blanket but as an electric blanket.

It may be calculated that for each molecule of triglyceride broken down to glycerol and fatty acids seven high-energy phosphate bonds would be needed to reconvert the fatty acids back to triglyceride, two for each of the three fatty acids and one to provide the glycerol phosphate. If three high energy phosphate bonds

are formed for each atom of oxygen consumed, then in this process a shade over one molecule of O_2 is needed per molecule of triglyceride synthesized. With all these hormones, maximum increases in O_2 consumption of the order of 2–3 μmol per 100 mg of tissue per hour are observed on the average under optimum conditions. Hence, to account for this amount of O_2 comsumption the turnover of triglyceride would have to also be in the range of 2–3 μmol per hour per 100 mg of tissue or 6–9 μmol of fatty acid would be re-esterified.

Adipose tissue from young animals when stimulated by adrenaline *in vitro* has been reported to release 5 μmol of fatty acid per 100 mg per hour.[52] A smaller release of fatty acid is observed when tissue from older animals is employed. It is worthy of note that the oxygen consumption of adipose tissue from young rats also exceeds that from older animals.[1, 8] Whether these facts are the reflection of a higher rate of fatty acid re-esterification and perhaps of the higher basal metabolic rate of young animals can not be answered at this time.

As indicated in Table 1, all the hormones which stimulate the release of fatty acids from adipose tissue and increase its oxygen consumption also enhance the breakdown of glycogen in this tissue. The glycogenolytic action of adrenaline, glucagon, and ACTH in other tissues has been traced to their ability to produce adenosine-3′,5′-phosphate.[53] The question has therefore been raised[53] as to whether the formation of this compound might be responsible for the stimulation by these hormones of both glycogen and fat breakdown in adipose tissue. On the other hand, the question might be asked: does the formation of fatty acids and their re-esterification lead to the production of adenosine-3′,5′-phosphate? Is it possible that the acyl-AMP compound formed during the conversion of fatty acids to fat acts as a potential source for the formation of the cyclic derivative of AMP? If such were the case, then the need for glycerol phosphate in the re-esterification process would be automatically ensured by stimulation of glycogen breakdown. It should be noted that serotonin stimulates the breakdown of glycogen in adipose tissue, but does not cause release of fatty acids.[21] This finding has been confirmed and in addition serotonin has been found to be without effect upon the O_2 comsumption of adipose tissue.[10] Hence increased glycogen breakdown per se does not necessarily stimulate O_2 uptake.

Summary.—Previous observations which show that hormones capable of accelerating the release of fatty acids from adipose tissue also stimulate the oxygen consumption of this tissue *in vitro* are extended. The list of hormones which possess this dual action now includes adrenalin, adrenocorticotropin, glucagon, thyrotropin, certain growth hormone preparations, the urinary fat mobilizing substance of Chalmers *et al.*, and the two pituitary peptides separated by Astwood and coworkers. Some of these compounds show a species specificity in their dual action. It is suggested that the stimulation of oxygen consumption is secondary to the liberation of fatty acids by these hormones and is a reflection of an increased consumption of high-energy phosphate for the re-esterification of these fatty acids. The results are discussed in relation to observations in the literature which support this premise and to the possible significance of this seemingly wasteful process in the generation of heat for the maintenance of body temperature.

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Abbreviations used are: ACTH (adrenocorticotropin), AMP (adenosine monophosphate), ADP (adenosine diphosphate), ATP (adenosine triphosphate), CoA (coenzyme A), F.M.S. (fat mobilizing substance), TSH (thyrotropic hormone), Pi (inorganic phosphate), and α-MSH (melanocyte stimulating hormone).

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*EXPERIMENTS ON HEMOGLOBIN BIOSYNTHESIS**

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We have been concentrating lately on the chemical characterization of the mechanism of peptide linking, the terminal reaction in the polymerization of amino acids. For this purpose, the laying down of peptide chains on ribosomes was studied with aminoacyl soluble ribonucleic acids (sRNA's), the "active" amino acids, as the donors. Unfortunately, in the much used systems from liver[1] and *E. coli*[2] the protein formed is rather ill-defined. In this predicament, we turned our attention to the hemoglobin-producing system in reticulocytes.[3] As is well known, these cells may be obtained from rabbits appropriately injected with phenylhydrazine. In the resulting phenylhydrazine anaemia, hemoglobin-producing, but already enucleated, red cell precursors, the reticulocytes, replace up to 90 per cent of the normal synthetically inert red cells. A hemoglobin-producing cell-free system has been prepared from reticulocytes;[4] it resembles those from liver and *E. coli*, with the important difference that it produces a well-defined protein.

After it had become clear that the aminoacyl-sRNA represents the activated amino acid from which the peptide link is formed, a rather amazing nonspecificity of aminoacyl-sRNA from various sources[5] aroused our interest. Apparently any amino acid-carrying sRNA would indiscriminately react with mammalian or microbial ribosomes; for example, it was reported[1] that ribosomes from rat liver or mouse neoplasm would utilize *E. coli*-derived aminoacyl-sRNA for synthesis of protein as easily as their own sRNA.

To confirm the general compatibility of any aminoacyl-sRNA, it was decided to attempt hemoglobin synthesis by cross-reacting *E. coli* aminoacyl-sRNA with rabbit reticulocyte ribosomes. Such experiments have been successful and it will largely be the purpose of this paper to report on them. Furthermore, a preliminary analysis is presented of the components of the aminoacyl-sRNA-ribosome system from reticulocytes.

Methods.—Preparation of E. coli aminoacyl-sRNA: Soluble-RNA from *E. coli* was prepared by the phenol procedure developed by Acs.[6] The bacterial paste was suspended in an equal volume of 0.001 *M* Tris-HCl buffer, pH 7.2, containing 0.01 *M* magnesium acetate. The heavy suspension was shaken with an equal volume of redistilled phenol at room temperature for at least 1 hour. The mixture was centrifuged at 10,000 × *g* for 30 minutes, the water layer was removed, and the phenol layer was washed once with 5 ml of 0.001 *M* Tris buffer, pH 7.2. To the combined water layers, one-tenth the volume of 20 per cent potassium acetate solution was added, and the