RNA biosynthesis in adipose tissue: effect of fasting

WILLIAM BENJAMIN and ALFRED GELLHORN

Department of Medicine and the Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N. Y.

ABSTRACT RNA metabolism has been examined in intact adipose tissue and isolated fat cells from rats.

The lipocyte contains three species of RNA with sedimentation rates corresponding to those of ribosomal and transfer RNA. The de novo biosynthesis of RNA by adipose tissue cells in vitro was demonstrated. The base ratios of the RNA formed indicate that it was synthesized from a DNA template.

Actinomycin D administered in vivo and in vitro decreased total RNA synthesis with the most marked effect on the synthesis of the heavy RNA components. Actinomycin D or puromycin added in vitro was not toxic: they did not inhibit total fatty acid biosynthesis or glucose utilization by the fat pad nor did they inhibit the immediate stimulation of fatty acid biosynthesis and glucose uptake by the addition of insulin in vitro.

Starvation for 48–72 hr significantly depressed the synthesis of the heavy RNA components as measured by in vitro uridine incorporation into the individual RNA classes. Refeeding the fasted rat with glucose repaired the defect in RNA biosynthesis *before* the biosynthesis of monoenoic fatty acid was completely restored. Actinomycin D administered at the time of refeeding prevented the repair of monoenoic fatty acid synthesis.

It is concluded that RNA metabolism is intimately involved in the control of biosynthetic reactions in adipose tissue.

KEY WORDS adipose tissue · isolated fat cells · rat · fasting · refeeding · stearate–oleate conversion · RNA biosynthesis · ribosomal RNA · sucrose density gradients · Actinomycin D · enzyme induction

THE OBSERVATIONS of Drury (1) and of Stetten and Boxer (2) on the changes in fatty acid biosynthesis in diabetes, and those of Rose and Shapiro (3) on the effects of starvation on fat metabolism, directed attention to adipose tissue as a metabolic organ. Since adipose tissue is exquisitely responsive to changes in the physiologic state of the whole organism (4) and its metabolism is characteristically affected by nutrition (5), hormones (6), and age (7) and because it is a highly differentiated tissue, it has become a useful model in the study of cell metabolism.

Recently, evidence has been obtained which indicates that control of fat cell metabolism is, in part, at the level of DNA-directed biosynthesis of RNA, for the administration of insulin to the diabetic rat induces the synthesis of enzymes concerned with the biosynthesis of saturated and monounsaturated fatty acids by stimulating the renewal of cellular RNA (8, 9). In the present studies, existing techniques have been modified for the determination of RNA metabolism in the intact adipose tissue and in the isolated fat cell to explore more precisely mechanisms operative in the control of fat cell metabolism. Starvation for 48–72 hr decreased the synthesis of the heavy RNA components of rat adipose tissue incubated in vitro and refeeding glucose to the starved animal restored RNA synthesis to normal.

MATERIALS AND METHODS

Male Wistar rats weighing between 140 and 170 g from the Columbia University colony were used in these experiments. They were fed rat pellets (A. E. Staley Manufacturing Co., Rockland, Ill.) ad libitum, or starved for 48–72 hr and refed with 10% glucose in the drinking water.

Incorporation of radioactive phosphorus into the ribonucleic acids of the epididymal fat pad was measured by incubating the tissue in modified Krebs-Ringer bicarbonate, pH 7.4 (without added phosphate ion), containing 3% bovine albumin (Sigma Chemical Co., St. Louis, Mo.) and 100–300 μc of ^{32}P (Radiochemical Centre, Amersham, England). Before use, the ^{32}P as orthophosphate in dilute HCl solution was heated at 100°C for 1 hr. Incubations were carried out at 37°C, gas phase 95% O_2–5% CO_2, in 4 ml of medium containing 50 μmoles of

glucose. After incubation the tissue was removed, washed in Krebs-Ringer bicarbonate solution, and frozen in dry ice until the extraction of RNA.

Isolated fat cells were prepared by the technique of Rodbell (10). All glassware was siliconized and solutions were maintained free from bacteria and fungus. Epididymal and lumbar fat from at least two animals was used for each assay. The tissue was incubated for 1 hr at 37°C in 4 ml of Krebs-Ringer bicarbonate–3% albumin, pH 7.4, containing 10 mg of collagenase (Nutritional Biochemical Corp., Cleveland, Ohio) and 10 μmoles of glucose. Fragments of tissue remaining after the procedure were removed manually and the cell suspension was centrifuged in a clinical centrifuge for 1 min. The debris and fluid were removed with a Pasteur pipette and the cells resuspended and washed 3 times with Krebs-Ringer bicarbonate solution. After the last wash the cells were resuspended in 8 ml of McCoy's tissue culture medium (11) containing 10% horse serum, glucose 12.5 mM, with the addition, per liter, of penicillin 200,000 units, tetracycline 2.5 mg, and streptomycin 40 mg. When ^{32}P was the label the tissue culture medium was formulated without added phosphate ion. Aliquots representing the cells from at least two animals were added to a 50 ml flask in a total volume of 8 ml. Flasks were incubated at 37°C in a slowly oscillating shaker, gas phase 95% air–5% CO_2. After 15 min of equilibration, 100 μc of uridine-5-T (24.4 c/mmole, Nuclear Chicago Corp.) was added. At the end of the incubation the cells were again recovered, the medium was discarded, and the cells were frozen in an acetone–dry ice bath.

The conversion of stearate to oleate by the fat pad was measured as described previously (12). Fatty acid biosynthesis and glucose uptake by the fat pad in the presence of crystalline zinc insulin (Eli Lilly Co., No.795372), actinomycin (Merck, Sharp, and Dohme), and puromycin (Nutritional Biochemical Corp., Cleveland, Ohio) were measured using techniques described elsewhere (8, 13). Glucose was measured by the glucose oxidase method (Glucostat, Worthington, Harrison, N.J.).

RNA was extracted by a modified phenol method of Click (14) and Sanger and Knight (15). The frozen tissue was placed in a Lourdes Homogenizer and homogenized at slow blade speed in the cold, with a solution in water-saturated phenol of 0.1 M glycine buffer pH 9.5, 0.1 M NaCl, 0.01 M EDTA with 0.2% bentonite (16), and 1% sodium deoxycholate (Difco Laboratories Inc., Detroit, Mich.) added. The phases were separated by centrifugation in the cold, the upper buffer phase was washed twice with cold diethyl ether, and the RNA was precipitated with 2 vol of cold 95% ethanol. The RNA was collected by centrifugation and the precipitate redissolved in buffer. The RNA was reprecipitated three times with ethanol. The final RNA precipitate was dissolved in buffer and centrifuged at 7500 × g for 5 min. Aliquots of the clear supernatant solution were assayed for radioactivity and for absorption at 260 mμ and 280 mμ, and analyzed by the sucrose density gradient technique (17). The RNA, layered over linear sucrose gradients 2–20% w/w in buffer, was centrifuged in a 25.1 ultracentrifuge head at 25,000 RPM in a Model L-2 Centrifuge (Beckman Instruments, Inc.) for 16–17 hr at 4°C. The gradients were analyzed using the Gilford spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio) to record ultraviolet absorbance at 260 mμ. Radioactivity of the purified RNA was determined on fractions which were precipitated with 5% trichloroacetic acid at 0°C. The precipitates were collected on Millipore filters (Millipore Filter Corp., Bedford, Mass.) and washed thoroughly. The filters were dried and placed in scintillation vials and counted in a Model 3003 Packard Liquid Scintillation Spectrometer using as phosphor 20% ethanol, 0.4% 2,5-diphenyloxazole, 0.04% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene.

Base analysis of the RNA was determined by the method of DeBellis (personal communication). The RNA of pooled peak tubes from the sucrose gradient analysis was precipitated and hydrolyzed in 0.33 N KOH at 37°C for 17 hr. An equimolar amount of perchloric acid was added at 0°C and the supernatant fraction saved after centrifugation. Nonradioactive AMP, CMP, GMP, and UMP were added and approximately 6 OD units were applied to thin-layer plates coated with Ecteola MN 300 (Brinkmann Co., Great Neck, N. Y.). Ascending chromatography was first carried out for 7 hr in isobutyric acid–0.5 M NH_4OH 10:6. To separate GMP from UMP ascending chromatography at a 90° angle to the first was carried out for 7 hr in *tert*-butyl alcohol–constant-boiling HCl–water 7:1.3:1.7. The spots were detected under UV light, scraped, and counted by suspending the powder in 4% Cab-O-Sil (Cabot Corp., Boston, Mass.) in phosphor.

RESULTS

The ribonucleic acids of rat epididymal fat as studied by sucrose gradient centrifugation (Fig. 1) were found to be composed of three species. The sedimentation coefficients and optical density patterns demonstrated the RNA to consist of two heavy and one light peak similar to rabbit reticulocyte RNA (18, 19). They are designated A (28S) and B (18S) for the heavier peaks and C (4S) for the lighter peak. ^{32}P-labeled RNA samples from peaks A and B migrated with ribosomal RNA obtained from the rabbit reticulocyte when centrifuged in a sucrose gradient. The two heavier components obtained from adipose tissue most probably correspond to ribosomal RNA, while the lightest component corresponded to transfer

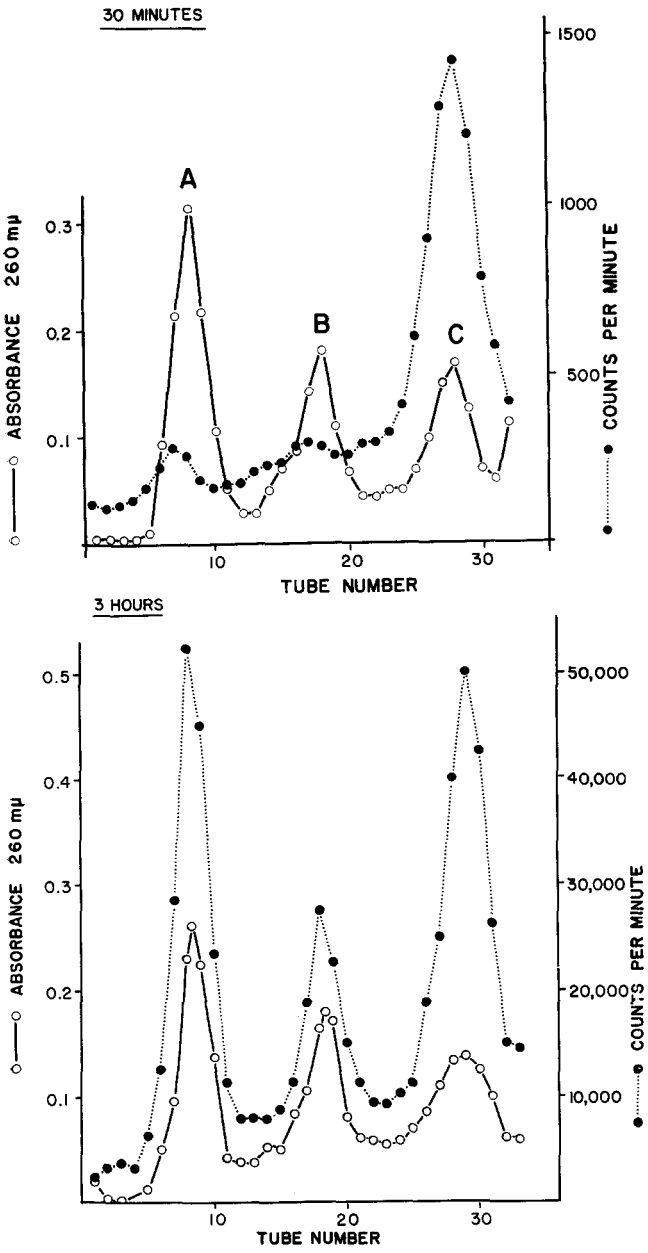

FIG. 1. The sedimentation of purified RNA from the rat epididymal fat pad labeled in vitro with ^{32}P for 30 min or 3 hr. Approximately 1 g of adipose tissue was incubated at 37 °C in 5 ml of Krebs-Ringer bicarbonate, 3% albumin, with 250 μc of carrier-free ^{32}P and 50 μmoles of glucose added. Isolation of the RNA and conditions of sucrose gradient centrifugation are described in the text.

RNA (20). Fig. 1 demonstrates the change, with time of incubation in vitro, in the pattern of incorporation of radioactive phosphorus into the ribonucleic acids of the rat epididymal fat pad. In short incubations, ^{32}P was found to be incorporated rapidly into the lightest component. With an increase in incubation time it was found that the incorporation of label into the acid-insoluble material precisely followed the heavy RNA peaks. By 3 hr of incubation, the incorporation of ^{32}P into the heavy RNA components exceeded the incorporation into the lightest component. The rate of incorporation of radioactive phosphorus into the ribonucleic acids (studied at 10 min, 30 min, and 1, 2, and 3 hr of incubation) was not constant.

To demonstrate that there was de novo biosynthesis of ribonucleic acid in the fat pad during incubation in vitro, samples from the peaks of each of the heavy RNA species were hydrolyzed with base and the bases separated by thin-layer chromatography. Radioactivity was found to migrate with the bases, and base ratios calculated by determining the counts in each base compared to incorporation into all the bases revealed base ratios similar to that reported by DeBellis (21) for rabbit reticulocyte RNA (Table 1).

Techniques were developed to study RNA biosynthesis in the isolated fat cell since the epididymal fat pad is a heterogeneous tissue composed of many different types of cells. The in vitro incorporation of uridine was measured both into the fat cell and the other cells of this tissue by incubation of the whole fat pad with uridine-5-T and then separating the cells. In two experiments, over 50% of the total amount of ribonucleic acid biosynthesized and of the total extracted RNA was found with the extraneous cells of the fat pad.

The rate of incorporation of uridine in vitro into the total RNA of the fat cell is shown in Fig. 2. After the first hour of incubation of cells in McCoy's medium, the incorporation of label into the RNA was linear with time. Fig. 3 shows the pattern of incorporation of uridine into the three species of ribonucleic acids of the isolated fat cell with time. After 1 hr of incubation the counts are correlated with the optical density pattern and with time they increase in each RNA peak. Isolated fat cells were incubated for 6 hr, then tritiated uridine was added and

TABLE 1 BASE ANALYSES OF ALKALINE HYDROLYSATES OF FAT CELL RIBOSOMAL RNA

		Nucleotide			
		AMP	CMP	GMP	UMP
		% of total ^{32}P			
Fat pad	28S(A)*	17.1	29.7	35.1	18.1
Fat pad cells	28S(A)	18.2	29.3	34.7	17.8
	18S(B)*	23.1	27.2	27.6	22.1
Isolated fat cells	28S(A)	20.9	27.1	34.0	18.0
	18S(B)	23.9	27.4	26.4	22.3

The purified RNA was isolated from either the whole fat pad or from fat cells isolated after the incubation of the fat pad with ^{32}P for 3 hr in Krebs-Ringer bicarbonate medium; or isolated fat cells were incubated with ^{32}P in McCoy's medium for 3 hr. The purified RNA was fractionated into 28S and 18S components by sedimentation in sucrose density gradients, and these were fractionated according to base components by thin-layer chromatography.

* Letters refer to peaks in Fig. 1.

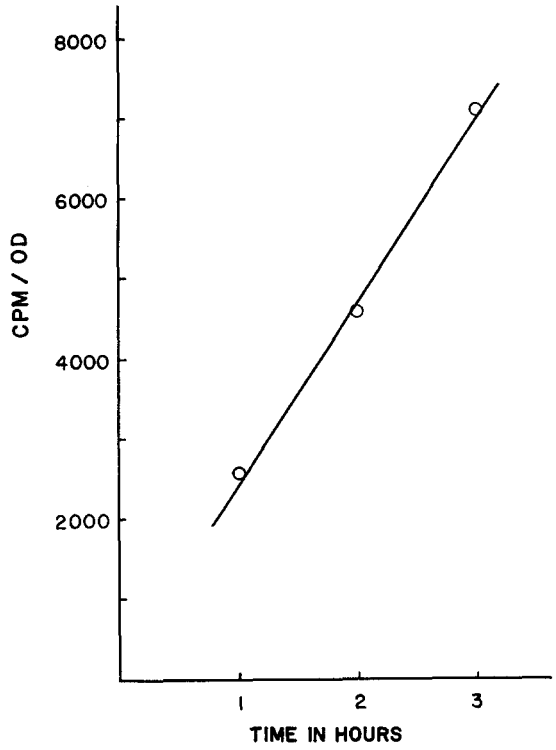

FIG. 2. Time course of appearance of uridine-5-T in the total purified RNA of the fat cell. Fat cells were incubated for various times in 8 ml of McCoy's medium, 12.5 mM in glucose, containing 100 μc of uridine, and the RNA was isolated as described in the text. Results are expressed as cpm/OD unit of RNA. In a series of ten determinations the isolated fat cell system incorporated 2030 cpm/OD unit per hr, SD 538.

the incubation carried out for an additional 2 hr. The rate of incorporation of uridine into the RNA was equal to that when the incubations were carried out over the first 2 hr, which indicates that the fat cell is viable for many hours in vitro.

It was necessary to determine whether the assay of RNA metabolism in the in vitro system of isolated fat cells was relevant to the behavior of adipose tissue in vivo.

TABLE 2 EFFECT OF ACTINOMYCIN D ON RNA BIOSYNTHESIS

^{32}P		Uridine-5-T		
			Actinomycin	
Control	Actinomycin in vitro	Control	in vitro	in vivo
3061*	1191	2046	181	362

Incorporation of ^{32}P into the RNA of the rat fat pad was assayed in vitro in 4 ml of Krebs-Ringer bicarbonate 3% albumin containing 200 μc of ^{32}P. Uridine incorporation into the RNA of isolated fat cells was assayed in vitro in 8 ml of McCoy's medium containing 100 μc of uridine. Actinomycin D in vitro, 5 μg/ml; in vivo, 15 μg/100 g body weight as described in the text.

* Results are expressed as counts per minute incorporated into the RNA per optical density unit per hour, and are the mean of two experiments.

Actinomycin D, 15 μg/100 g body weight, was injected intraperitoneally either 3 hr prior to assay or every 12 hr for two doses, the assay being carried out 12 hr after the last dose. In Table 2 is shown the incorporation of ^{32}P and uridine into the RNA of adipose tissue after treatment with Actinomycin D either in vivo as above or in vitro (5 μg/ml). Both treatments markedly decreased in vitro RNA biosynthesis. Sucrose gradient analysis demonstrated that the synthesis of heavy RNA by fat cells was almost abolished by treatment with Actinomycin D, while biosynthesis of the lightest RNA was markedly reduced (Fig. 4 and 5).

To determine whether Actinomycin D was nonspecifically toxic to adipose tissue, the epididymal fat pad was incubated with the drug, and fatty acid biosynthesis and glucose uptake were measured (Table 3). The effect of puromycin on adipose tissue was included as an appropriate control. The results demonstrate that over the 30 min to 2 hr assay period these toxic antibiotics did not significantly decrease fatty acid biosynthesis nor did they affect the stimulation of glucose uptake and fatty acid biosynthesis by insulin. These observations confirm the specificity of action of Actinomycin D (22) and puromycin (23) in the doses employed and permit the con-

TABLE 3 EFFECT OF INSULIN ON ADIPOSE TISSUE IN THE PRESENCE OF ACTINOMYCIN D OR PUROMYCIN

		Acetate Incorporation*					Glucose Uptake			
Tissue	Incubation Time	Control	Actino-mycin	Puromycin	Insulin + Control	Insulin + Actino-mycin	Insulin + Puromycin	Insulin + Control	Insulin + Actino-mycin	Insulin + Puromycin
	min	*umoles incorporated into lipids per g tissue per hr*						*umoles per g tissue per hr*		
Normal I	30	1.84	1.60	1.58	3.04	3.68	3.00	16.2	15.9	24.1
Normal II	30	1.74	2.28	1.86	2.56	3.64	3.14	18.5	17.1	12.1
Normal III	120	1.74	1.94	1.83	6.05	4.76	5.15	15.6	16.6	18.7
Diabetic I	120	0.12	0.18	0.09	0.80	0.71	0.76			
Diabetic II	120	0.60	0.89	0.31	2.57	3.54	2.95			

Paired adipose tissues, 50–150 mg of each, were incubated at 37 °C in Krebs-Ringer bicarbonate media, with 3% bovine albumin added to a total volume of 4 ml. Flasks preincubated for 30 min with Actinomycin D 5 μg/ml or puromycin 270 μg/ml. Insulin 0.1 unit/ml.

* Mean results of individual experiments performed in duplicate.

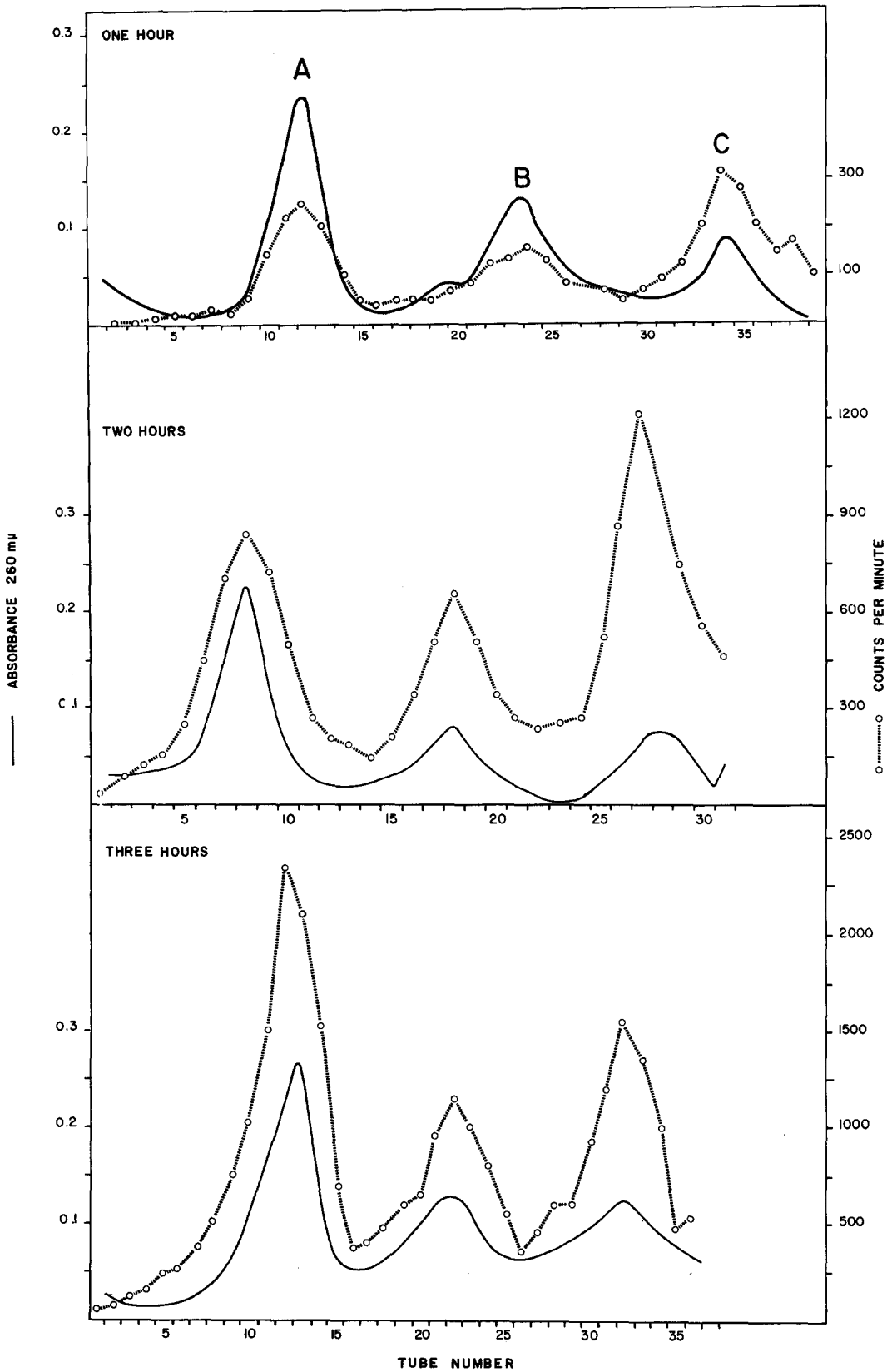

FIG. 3. Sedimentation of purified RNA from the isolated fat cell incubated with uridine-5-T in McCoy's medium for varying times. Fat cells from approximately 5 g of adipose tissue from rats weighing 150 g were prepared by the collagenase method (10). Incubation, isolation, and centrifugation conditions described in the text.

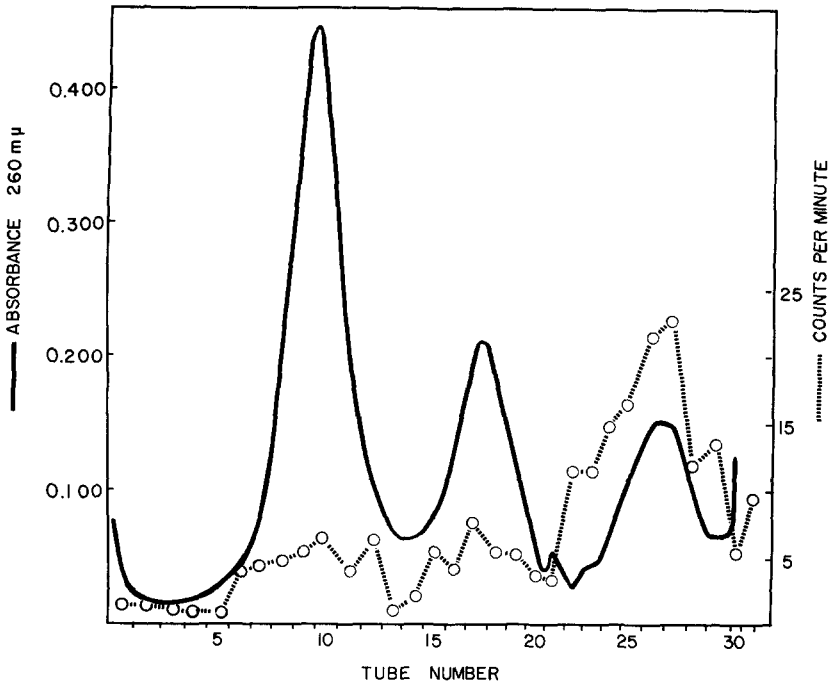

FIG. 4. Sedimentation of purified RNA from isolated fat cells incubated in vitro for 2 hr with Actinomycin D, 5 μg, with 100 μc of uridine-5-T. For control, see 2 hr sample, Fig. 3.

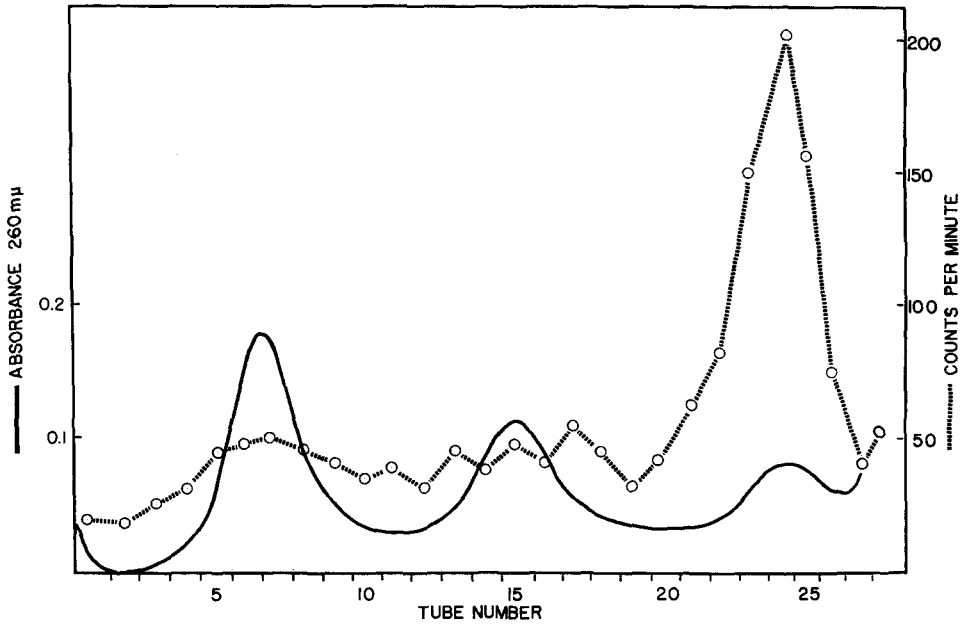

FIG. 5. Sedimentation of purified RNA from isolated fat cells obtained from rats treated with Actinomycin D. Actinomycin D, 15 μg/100 g body weight, was administered intraperitoneally 24 and 12 hr prior to sacrifice. Incubation for 2 hr in McCoy's medium containing 100 μc of uridine-5-T. For control, see 2 hr sample, Fig. 3.

clusion that they are not nonspecifically toxic to adipose tissue in vitro.

Since adipose tissue is markedly sensitive to the state of nutrition of the animal, the effect of starvation and glucose refeeding on RNA metabolism and the conversion of stearate to oleate was studied. Figs. 6 and 7 show the marked inhibitory effect of fasting on ribosomal RNA synthesis in isolated fat cells incubated for 30 min or 3 hr. Total count incorporation per optical density unit was not significantly decreased by fasting because

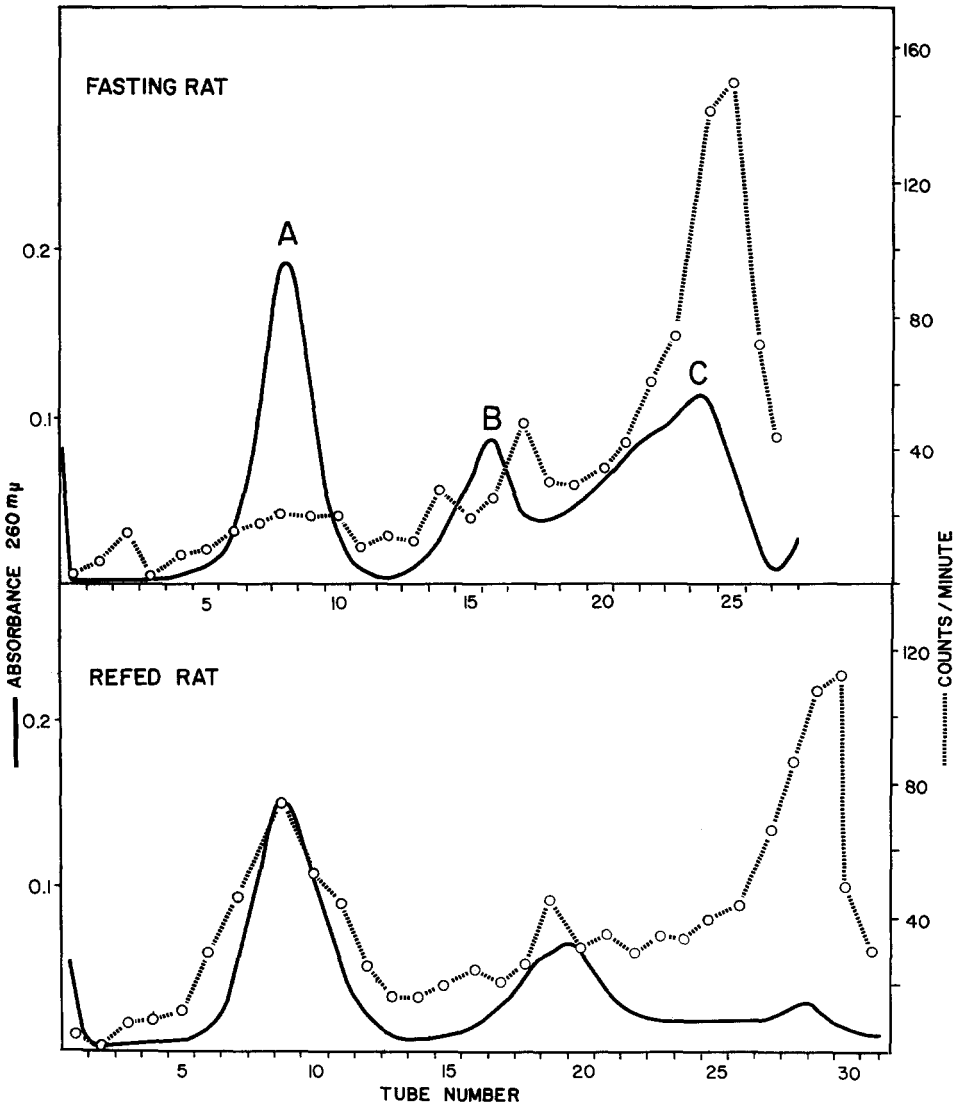

FIG. 6. Sedimentation of purified RNA from the isolated fat cells of fasted and refed rats. Animals were fasted for 48 hr and refed with 10% glucose in their drinking water for 4 hr. Cells were incubated in McCoy's medium for 30 min with 100 μc of uridine-5-T. Isolation and centrifugation of the purified RNA as described in the text.

incorporation of label associated with peak C (light RNA) was not depressed by starvation. This number must be viewed with caution as it is very likely that much of the radioactive RNA that sediments in the light peak represents breakdown products and perhaps incomplete products of heavier material. However, it was found that the incorporation of label into the heavy region of the gradient was depressed and heterogeneous with respect to the optical density peaks A and B. The synthesis of stearate to oleate was depressed in the tissue from the fasted animal (Table 4). The restoration of heavy RNA synthesis to normal following 4–7 hr of refeeding with glucose in the drinking water is shown in Fig. 6 and 7; oleate synthesis was partially repaired by this refeeding (Table 4). It appears, then, that restoration of the synthe-

TABLE 4 CONVERSION OF STEARIC ACID TO OLEIC ACID BY RAT EPIDIDYMAL FAT PAD

	Incorporation of radioactivity	Conversion of stearate to oleate
	cpm/hr/mg wet tissue	%
Control	8470	100
Fasted	1789	12*
Refed	2789	39*

Tissue (75–150 mg) was incubated for 1 hr in Krebs-Ringer bicarbonate–3% bovine albumin, with 1 μmole of stearic acid in 0.1 ml of propylene glycol containing 10 μc of stearic acid-1-^{14}C and 50 μmoles of glucose in 4 ml total volume. Animals were fasted for 48–72 hr and refed with 10% glucose in their drinking water for 4–7 hr. Results are the mean of three experiments.

* Compared to contemporaneous controls.

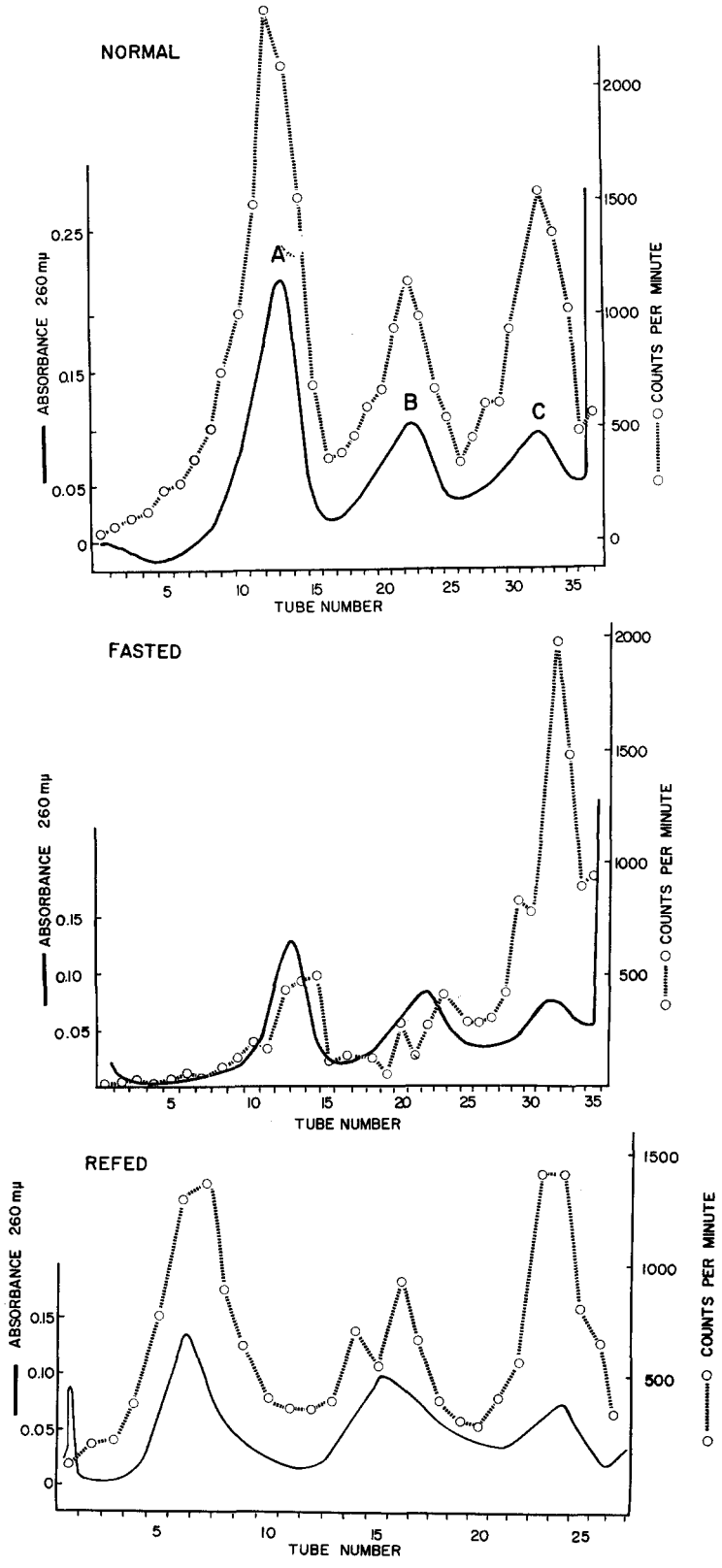

FIG. 7. Sedimentation of purified RNA from the isolated fat cells of normal, fasted, and refed rats. Animals were fasted for 72 hr and refed with 10% glucose in their drinking water for 7 hr. Incubation in McCoy's medium for 3 hr with 100 μc of uridine-5-T.

TABLE 5 EFFECT OF ACTINOMYCIN D ON CONVERSION OF STEARIC ACID TO OLEIC ACID BY RAT EPIDIDYMAL FAT PAD

	Incorporation of Radioactivity	Olefin Synthesis*
	cpm/hr/mg wet tissue	%
Control	1885	55
Fasted	1016	3
Refed	3699	35
Actinomycin-treated, refed	2325	10

Tissue (200–300 mg) from 100–130 g rats was incubated for 1 hr in Krebs-Ringer bicarbonate–3% bovine alumin with 0.5 μmole of stearic acid in 0.1 ml. of propylene glycol containing 5 μc of stearic acid-1-^{14}C and 100 μmoles of glucose in 4 ml total volume. Results are the mean of at least two experiments.

* Percentage conversion of stearate to oleate by adipose tissue.

Control, animals fed ad libitum.

Fasted, animals fasted for 48 hr.

Refed, animals fasted for 36 hr then refed ad libitum for 12 hr with 5% dextrose in the drinking water.

Actinomycin-treated refed, animals fasted 36 hr, received Actinomycin D intraperitoneally (20 μg/100 g), and refed as above.

sis of heavy RNA preceded the repair of the enzyme system.

To determine whether Actinomycin D administered to the fasted animal at the time of refeeding would prevent the repair of monoene synthesis in adipose tissue the following experiment was performed (Table 5). The conversion of stearate to oleate was measured in adipose tissue obtained from four groups of animals (normal, fasted, refed, and actinomycin-treated refed). At the time of sacrifice the stomach contents were examined to confirm that the refed and actinomycin-treated refed animals had eaten. The results demonstrate that a single injection of Actinomycin D effectively halts the repair of the monoene enzyme system by refeeding.

DISCUSSION

Morphological evidence reported by Sheldon has demonstrated that the lipocyte contains ribonucleoprotein particles on a well organized endoplasmic reticulum (24). In this study, adipose tissue was found to contain the three species of ribonucleic acids characteristic of mammals (18). They consist of two classes of heavy molecules, probably part of the structure of the ribosome, and a lighter moiety classified as transfer RNA. When adipose tissue or the isolated cells were incubated in vitro in Krebs-Ringer bicarbonate or McCoy's medium with carrier-free radioactive phosphorus, active incorporation of the label into the ribonucleic acids of the cell was found. The rate of incorporation of ^{32}P into the adipose tissue RNA was not linear. The lag period may represent impairment of diffusion of the phosphate ion into the cell, or the time required for the label to be covalently linked to the 5′-position of the nucleotides which are the immediate precursors of RNA. At short incubation times, the distribution of incorporated label in the density gradient centrifugation pattern was not correlated with the optical density peaks corresponding to the three RNA species. This is analogous to the findings of Di-Girolamo, Henshaw, and Hiatt in studies of the liver cell (25). After 2–3 hr of incubation, however, the peaks of radioactivity coincided exactly with the heavy and light RNA peaks (Fig. 1).

After in vitro incubation of the fat pad or the lipocyte with ^{32}P, base analysis of the individual RNA classes was carried out. The finding that the content of guanine nearly equals cytosine and adenine equals uridine is suggestive of a high degree of secondary structure based upon the Watson-Crick (26) type of base pairing. The observation that the guanine plus cytosine content is greater than that of adenine plus uridine is characteristic of mammalian RNA (21). The results of the base analysis prove that the fat pad and the isolated fat cell in vitro synthesize RNA de novo. These observations, coupled with the finding that Actinomycin D in vivo or in vitro specifically inhibits RNA synthesis, indicate that the RNA synthesized is made from a DNA template.

Because adipose tissue is so responsive to homeostatic controls of the host and because of its apparent cellular homogeneity, it has seemed to be an excellent model system for the study of mechanisms of cell control. The recent reports of Rodbell have, however, emphasized that even this apparently uniform tissue has a large admixture of cells unrelated to the lipocyte (10). Using collagenase to free the lipocyte from its matrix, Rodbell demonstrated that the isolated fat cell retained the characteristics of the intact tissue with regard to the major pathways of glucose and fatty acid metabolism. We have adopted Rodbell's methods, with slight modification, and have shown that incorporation of uridine into RNA of the lipocyte is linear with time and that all three species of RNA are synthesized. It is noteworthy that when the intact tissue was incubated with uridine and the epididymal fat pad then disrupted with collagenase, approximately 50% of the incorporation into RNA was into cells other than lipocytes.

The synthesis of heavy RNA was inhibited by actinomycin to a greater extent than that of the lightest RNA, as Revel and Hiatt also noted in studies with liver (27). These results indicate that the metabolic functional state of the cell in vivo is reflected in its RNA biosynthetic rate in vitro. The observations also show that the biosynthesis of RNA was decreased by actinomycin under the identical conditions employed by Gellhorn and Benjamin (8) to demonstrate the inhibition by actinomycin of insulin induction of enzymes in vivo. The effect of Actinomycin D on RNA synthesis was shown not to be due to a nonspecific toxicity of the antibiotic by demonstrating that fatty acid biosynthesis from acetate in vitro, and the

stimulation of glucose uptake by insulin, were not affected by the presence of Actinomycin D (or of puromycin). The immediate stimulation of glucose uptake by insulin in vitro is not, then, mediated by new protein or RNA synthesis. It should be stressed that the immediate effect of insulin in vitro is only one facet of its action on cell metabolism.

In response to starvation, adipose tissue mobilizes its stored triglyceride in the form of free fatty acids and decreases fatty acid biosynthesis and glucose utilization. The alterations in lipid metabolism of adipose tissue during fasting resemble those occurring in diabetes, and this suggested that they might be related to modifications in RNA biosynthesis. In this study it was found (Table 4) that the restoration of monoene synthesis is incomplete in the adipose tissue from starved rats after the administration of glucose for 7 hr. Since other studies on the repair of total fatty acid and individual fatty acid biosynthesis in the diabetic (8, 9, 12, 28)demonstrated that time was necessary for the repair, this suggested that new enzyme synthesis might be required. It was pertinent, therefore, to determine whether or not fasting had an effect on RNA metabolism and whether continued RNA synthesis was necessary for the repair of monoene synthesis during refeeding. Fasting induced a consistent decrease in heavy RNA biosynthesis by fat cells, as measured by uridine incorporation into the individual classes of RNA. Those counts which were incorporated into acid-insoluble material from the portion of the density gradient tube normally containing the heavy RNA were distributed heterogeneously, which suggests that when heavy RNA synthesis is depressed in this system, messenger RNA is being synthesized. The defect in RNA synthesis was repaired after only 4 hr of glucose refeeding, at a time when biosynthesis of monoenoic acid was still markedly depressed. This suggested that a very early step in the repair of monoenoic fatty acid biosynthesis by the fat cell after starvation is the synthesis of the ribosomal complex and, presumably, the specific informational RNA leading to the synthesis of specific enzyme proteins. The administration of Actinomycin D at the time of refeeding prevented the repair of monoene synthesis, thus confirming the need for continued RNA synthesis in the repair process.

The mechanism whereby starvation or administration of glucose controls the synthesis of the heavy RNA components is as yet unknown.

We wish to express our gratitude to Drs. Robert H. DeBellis and Robert Click for their instruction in nucleic acid chemistry. We also wish to thank Dr. Sam Graff and Mr. Robert Rothstein for their aid in tissue culture techniques.

This work was supported in part by grants from the National Cancer Institute, U. S. Public Health Service (CA 02332), and the Alma Toorock Memorial.

Manuscript received 23 August 1965; accepted 17 November 1965.

References

1. Drury, D. R. *Am. J. Physiol.* **131:** 536, 1940.
2. Stetten, D., Jr. and G. E. Boxer. *J. Biol. Chem.* **156:** 271, 1944.
3. Rose, G., and B. Shapiro. *Biochim. Biophys. Acta* **18:** 504, 1955.
4. Wertheimer, H. E. In *Adipose Tissue—Handbook of Physiology*, edited by A. E. Renold and G. Cahill, Jr. Williams & Wilkins Co., Baltimore, 1965, p. 5.
5. Hausberger, F. X., and S. W. Milstein. *J. Biol. Chem.* **214:** 483, 1955.
6. Vaughan, M. *J. Lipid Res.* **2:** 293, 1961.
7. Benjamin, W., A. Gellhorn, H. Kundel, and M. Wagner. *Am. J. Physiol.* **201:** 540, 1961.
8. Gellhorn, A., and W. Benjamin. *Science* **146:** 1166, 1964.
9. Gellhorn, A., and W. Benjamin. *Ann. N. Y. Acad. Sci.* **131:** 344, 1965.
10. Rodbell, M. *J. Biol. Chem.* **239:** 375, 1964.
11. Hsu, T. C., and O. S. Kellogg, Jr. *J. Natl. Cancer Inst.* **25:** 221, 1960.
12. Benjamin, W., and A. Gellhorn. *J. Biol. Chem.* **239:** 64, 1964.
13. Gellhorn, A., W. Benjamin, and M. Wagner. *J. Lipid Res.* **3:** 314, 1962.
14. Click, R. E. The role of nucleic acid protein synthesis in respiration and growth of plant tissues. *Doctoral Dissertation*, University of California, 1964.
15. Sanger, H. L., and C. A. Knight. *Biochem. Biophys. Res. Commun.* **13:** 455, 1963.
16. Fraenkel-Conrat, H., B. Singer, and A. Tsugita. *Virology* **14:** 54, 1961.
17. Britten, R. J., and R. B. Roberts. *Science* **131:** 33, 1960.
18. Hall, B. D., and P. Doty. *J. Mol. Biol.* **1:** 111, 1959.
19. T'so, P. O. P., and R. Squires. *Federation Proc.* **18:** 341, 1959.
20. Spirin, A. S. In *Progress in Nucleic Acid Research*, edited by J. N. Davison and W. E. Cohn. Academic Press, New York, 1963, p. 201.
21. DeBellis, R. H., N. Gluck, and P. Marks. *J. Clin. Invest.* **43:** 1329, 1964.
22. Reich, E., R. M. Franklin, A. J. Shatkin, and E. L. Tatum. *Proc. Natl. Acad. Sci. U.S.* **48:** 1238, 1962.
23. Yarmolinsky, M. B., and G. L. de la Haba. *Proc. Natl. Acad. Sci. U.S.* **45:** 1721, 1959.
24. Sheldon, H. In *Adipose Tissue—Handbook of Physiology*, edited by A. E. Renold and G. Cahill, Jr. Williams & Wilkins Co., Baltimore, 1965, p. 25.
25. DiGirolamo, A., E. C. Henshaw, and H. H. Hiatt. *J. Mol. Biol.* **8:** 479, 1964.
26. Watson, J. D. *Science* **140:** 17, 1963.
27. Revel, M., and H. H. Hiatt. *Biochem. Biophys. Res. Commun.* **17:** 730, 1964.
28. Gellhorn, A., and W. Benjamin. *Biochim. Biophys. Acta* **84:** 167, 1964.