perceived visually in fluorescence microscopy is insufficient for positive identification of a product. The introduction of interference filters reduces the artifact of unequal color perception by the eye and provides increased selectivity without serious loss in sensitivity. Such a maneuver is convenient for separating the fluorescence of the formaldehyde derivatives of norepinephrine and 5HT in tissues where they occur together, such as the pineal body (*11*), and for separating the fluorescence of the formaldehyde derivative of histamine from the other derivatives. Photometric measurement of emitted fluorescent light permits a quantitative estimate of identifiable biogenic amines seen in fluorescence microscopy.

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References and Notes

1. D. Lagunoff, M. Phillips, E. P. Benditt, *J. Histochem. Cytochem.* **9**, 534 (1961).
2. B. Falck, *Acta Physiol. Scand.* **56**, Suppl. 197 (1962); A. Carlsson, B. Falck, N-Å. Hillarp, *ibid.*, Suppl. 196 (1962).
3. J. Adams-Ray, A. Dahlström, K. Fuxe, N-Å. Hillarp, *Experientia* **20**, 80 (1964).
4. The formaldehyde condensation products are believed to be the following: norepinephrine, 1,6,7-trihydroxy-3,4-dihydro isoquinoline (*5*); 5HT, 6-hydroxy-3,4-dihydro-β-carboline (*5*); histamine, tetrahydropyrido-3,4-iminazole [see *1*, and H. H. Dale and H. W. Dudley, *J. Pharm. Exptl. Therap.* **18**, 103 (1921)].
5. While the fluorescence characteristics of the histamine-formaldehyde derivative have not been published, those of the catecholamine- and 5HT-derivatives have been given by H. Corrodi, and N-Å. Hillarp, [*Helv. Chim. Acta*, **46**, 2435 (1963)] as 240. 305, 355 mμ/470 mμ and 390-410/510-520, respectively, presumably uncorrected values (excitation maxima/emission maxima).
6. B. Chance and V. Legallais, *Rev. Sci. Instr.* **30**, 732 (1959); R. A. Olson, *ibid.* **31**, 844 (1960); L. N. M. Duysens and J. Amesz, *Biochim. Biophys. Acta* **24**, 19 (1957); C. S. French, in *Luminescence of Biological Systems*, F. H. Johnson, Ed. (AAAS, Washington, D.C., 1955).
7. M. Ritzén, *Proc. 2nd Intern. Congr. of Histochemistry and Cytochemistry*, Frankfurt, Germany (1964), abstracts, p. 187.
8. Microscopic spectrofluorometry was carried out with a Leitz Ortholux microscope with high pressure mercury light source (HBO 200) and dark-field condenser. Exciting light was filtered through one of the following filters: (i) a Schott UG-1, 2 or 4 mm, which passes the 365 mμ Hg line, and lower wavelengths, (ii) a Baird interference filter passing the 405 mμ Hg line, or (iii) a Baird interference filter passing the 436 mμ Hg line. The barrier filter slide was modified to accept a number of interference filters (Bausch and Lomb Inc., second order series, half-band width 5–6 mμ, recalibrated by us to ±1 mμ peak transmission) of wavelengths from 400 to 620 mμ. An RCA 1P21 photomultiplier tube was mounted in a Leitz microscopic photometer attachment and connected to an Aminco photometer. The field recorded by the phototube corresponded to a circular area of 17 or 23 μ diameter in the object plane, the approximate diameter of the cells observed. A Corning 3-73 or 3-72 sharp-cutoff filter served to remove any scattered blue or ultraviolet light passed through the third order peak of the interference-type barrier filters. The instrument was calibrated for spectral response of its 1P21 phototube and barrier filters by means of tungsten light of 3200°K and a spectral energy distribution curve for this color temperature. Photometer readings of the fluorescent light emitted from single cells were made immediately on exposure of the preparation to exciting light and the readings recorded as "relative fluorescent intensity" (the product of the photometer scale reading in percentage transmittance and the sensitivity multiplier switch setting), corrected for the phototube response and barrier filter transmission when emission spectra were being recorded. The "blank" correction for background fluorescence and light scatter was made by subtracting the reading taken from nonfluorescent cells in the same preparation, or an average of readings taken from cells in the control preparation (without formaldehyde treatment). A more complete description of this method is in preparation.
9. D. Lagunoff, personal communication.
10. G. R. S. Carlini, G. A. Fischer, N. J. Giarman, *J. Pharm. Exptl. Therap.* **146**, 74 (1964).
11. Å. Bertler, B. Falck, C. Owman, *Kungl. Fysiograf. Sällskapets Lund Förh.* **33**, 13 (1963).
12. We thank Dr. Britton Chance of the Johnson Foundation, University of Pennsylvania and Dr. Peter Bartels, E. Leitz, Inc., New York, for assistance with instrumentation, Dr. Bengt Falck, Department of Histology, University of Lund, Sweden and Dr. David Lagunoff, Department of Pathology, University of Washington School of Medicine, for histochemical information, and Dr. Glenn Fischer, Department of Pharmacology, Yale University School of Medicine, for advice on tissue culture methods. The study was supported by USPHS grant NB-00940.

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Epinephrine-Induced Normalization of Lipid Metabolism in Adrenalectomized Rats

Abstract. *In adrenalectomized rats given adequate supportive therapy with sodium chloride and hydrocortisone, lipemia is markedly elevated after a triglyceride meal. This defect can be corrected by the administration of epinephrine.*

Recently we observed (*1*) that patients with Addison's disease exhibited postprandial delay in the rate of decrease in the concentration of plasma triglyceride, even though these patients were adequately supplied with hydrocortisone and desoxycorticosterone acetate. A similar delay also occurs in subjects with coronary artery disease (*2*) and in healthy subjects particularly prone to coronary artery disease (*3*). This prolonged elevation of the concentration of plasma triglyceride is surprising since absorption of fat is deficient in both man (*4*) and animals (*5*) suffering from untreated adrenal insufficiency. Our observation suggested that patients with Addison's disease, who apparently received satisfactory treatment, still lacked some adrenal hormone(s) (other than hydrocortisone and desoxycorticosterone acetate) associated with adequate lipid transport or disposition. To investigate this possibility we subjected adrenalectomized rats to a series of studies.

In the first study, four groups of adult male rats (Long-Evans strain), weighing approximately 300 g each, were adrenalectomized. The first group of ten adrenalectomized rats received subcutaneous injections of 0.075 mg of epinephrine in oil at approximately 9:00 a.m. and again at 4:00 p.m.; the second group of 12 received 0.5 mg of hydrocortisone subcutaneously twice each day at similar times; the third group of 13 received both drugs subcutaneously twice each day; while the fourth group of 6 rats received no drugs. All adrenalectomized rats were given 0.8 percent NaCl to drink. Seventy-two hours after adrenalectomy all rats were given 3 ml of cottonseed oil by stomach tube, the abdominal segment of each thoracic duct was cannulated by a previously described technique (*6*), and intestinal lymph from each rat was collected separately for 3 hours and analyzed for triglyceride content (*7*). For control purposes, sham operations were performed on 17 rats; 72 hours later, after 12 of these rats had received 3 ml of oil, the lymph ducts of all 17 were cannulated, and lymph was collected for 3 hours and analyzed for triglyceride (*7*). During the studies all rats were housed in a quiet room isolated from ordinary laboratory activity except for the time during which samples of lymph or blood were collected.

In the second study three groups of adrenalectomized rats, which were also given 0.8 percent NaCl to drink, were injected with epinephrine (0.075 mg twice each day), hydrocortisone (0.5 mg twice each day), or both. A fourth group of adrenalectomized rats received no drugs. For control purposes four groups of rats were given similar treatment after sham operations. All

groups were given 3 ml of cottonseed oil by stomach tube 72 hours after adrenalectomy or sham operation. Blood samples (1.5 ml) obtained by snipping the tail were collected in heparin-containing tubes before, and 6 hours after, the oil was given, were analyzed for plasma triglyceride, and in most cases were also analyzed for free fatty acids according to the method of Dole (*8*). In addition, blood samples were obtained from the adrenalectomized rats receiving hydrocortisone and from the untreated control rats 24 hours after the oil was given.

The absorption of fats in adrenalectomized rats is shown in Table 1. Alert appearance and other indications that adrenalectomized rats were in excellent condition during the collection period were observed only in the group that received both epinephrine and hydrocortisone.

Despite the marked impairment in intestinal absorption of fat in untreated adrenalectomized rats or in adrenalectomized rats treated with epinephrine only, the postprandial increase in the concentration of triglyceride in the plasma (see Table 2) was as great as that observed in the control rats. Moreover, when intestinal absorption of fat was improved in adrenalectomized rats by administration of hydrocortisone and the general condition of these rats appeared excellent, a new defect in their fat metabolism became apparent: the postprandial concentration of plasma triglyceride increased much more and remained higher than that of the sham-operated control rats, regardless of whether the controls had been treated with the same corticosteroid or not (Table 2). However, when the adrenalectomized rats received both hydrocortisone and epinephrine, the postprandial concentrations of triglyceride in the plasma approximated those of the controls. The general condition of all adrenalectomized rats was excellent during this phase of the study.

These results suggest that in the adrenalectomized rat, seemingly well maintained with NaCl and hydrocortisone, there is a very marked postprandial derangement in the metabolism of triglyceride, and that the derangement resembles that in patients with Addison's disease who have received similar treatment (*1*). Epinephrine prevented or reversed the defect—a rather surprising property for this hormone to possess and one not known heretofore. The prevention or reversal of the defect is due specifically to epinephrine and not merely to a general improvement in the animals, since the general condition of all rats was excellent during this part of the study.

Table 1. Intestinal absorption of fats in adrenalectomized rats. Values are means ± standard error of the mean.

No. of rats	Average wt. (g)	Lymph vol. (ml)	Intestinal lymph triglyceride (mg/100 ml)	Intestinal lymph triglyceride (mg/3 hr)
		Untreated adrenalectomized rats		
6	303	2.0 ± 0.4	1145 ± 140	20.5 ± 5.1
		Adrenalectomized rats given epinephrine		
10	302	3.0 ± 0.4	770 ± 77	24.3 ± 4.3
		Adrenalectomized rats given hydrocortisone acetate		
12	294	2.54± 0.3	1413 ± 105	46.0 ± 3.2
		Adrenalectomized rats given epinephrine and hydrocortisone acetate		
13	295	4.4 ± 0.4	1628 ± 112.0	70.9 ± 7.4*
		Normal rats		
12	293	3.8 ± 0.2	1997 ± 98	76.2 ± 5.8*
		Normal rats (starved)†		
5	288	4.1 ± 0.5	357 ± 29	14.8 ± 1.9

* These two values differ significantly ($p < .001$) from the unmarked values in the same vertical column. † Received no oil before intestinal lymph was collected.

Table 2. Concentrations of triglyceride and free fatty acids in plasma of adrenalectomized rats before and after the administration of cottonseed oil. Values are means ± standard error of the mean.

No. of rats	Average wt. (g)	Triglyceride (mg/100 ml)			Free fatty acids (μeq/liter)	
		Before admin. of oil	6 hr after admin. of oil	24 hr after admin. of oil	Before admin. of oil	6 hr after admin. of oil
		Untreated adrenalectomized rats				
16	262	10 ± 4.1	52 ± 5.2			
		Adrenalectomized rats given epinephrine				
10	292	9 ± 2.1	66 ± 13.8		805 ± 155	1235 ± 160
		Adrenalectomized rats given hydrocortisone acetate				
15	297	11 ± 3.4	198 ± 18.2*	60 ± 23	661 ± 52	3302 ± 150
		Adrenalectomized rats given epinephrine and hydrocortisone acetate				
20	307	7 ± 2.0	48 ± 7.5		1154 ± 232	1317 ± 129
		Control rats				
16	312	10 ± 6.0	50 ± 5.0	14 ± 2	1031 ± 140	2476 ± 324
		Control rats given epinephrine				
10	298	11 ± 2.8	51 ± 7.8			
		Control rats given hydrocortisone acetate				
16	301	12 ± 5.0	58 ± 6.0		877 ± 130	2428 ± 200
		Control rats given epinephrine and hydrocortisone acetate				
10	298	9 ± 4.1	29 ± 6.1		1117 ± 125	1631 ± 180

* This value differs significantly ($p < .001$) from all other values in the same vertical column.

Although epinephrine also appeared to improve the absorption of fats by adrenalectomized rats treated with hydrocortisone, as measured by analysis of fat in intestinal lymph, we hesitate to say that this improvement in absorption is due to some additional specific action of epinephrine, because the administration of epinephrine without hydrocortisone did not improve absorption. Instead we believe it probable that administration of epinephrine kept the general condition of the adrenalectomized rats that had been given both hydrocortisone and epinephrine from deteriorating during the intubation of their intestinal lymph ducts. This in turn insured a good flow of lymph and a consequent normal absorption of triglyceride. It has been our experience (*9*) that the intestinal absorption of lipid cannot proceed normally if the animal is in poor general condition during the collection period.

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References and Notes

1. M. Friedman, S. O. Byers, R. H. Rosenman, *J. Am. Med. Assoc.* **190**, 959 (1964).
2. S. J. Thannhauser and M. M. Stanley, *Trans. Assoc. Am. Physicians* **62**, 245 (1949); R. H. Seller, J. Brachfeld, H. Sandberg, S. Bellet, *Am. J. Med.* **27**, 231 (1959).
3. M. Friedman, R. H. Rosenman, S. O. Byers, *Circulation* **29**, 874 (1964).
4. D. J. McBrien, R. V. Jones, B. Creamer, *Lancet* **1963-I**, 25 (1963).

5. R. L. Hakkila and P. I. Halonen, *Ann. Med. Exp. Biol. Fenniae (Helsinki)* **31**, 36 (1953); D. J. Ingle, *J. Clin. Endocrinol.* **3**, 603 (1943).
6. M. W. Biggs, M. Friedman, S. Byers, *Proc. Soc. Exptl. Biol. Med.* **78**, 641 (1951).
7. E. Van Handel and D. G. Zilversmit, *J. Lab. Clin. Med.* **50**, 152 (1957).
8. V. P. Dole and H. Meinerts, *J. Biol. Chem.* **235**, 2595 (1960).
9. S. O. Byers and M. Friedman, *Am. J. Physiol.* **192**, 427 (1958).
10. Supported by grants from PHS, HE-00119 and HE-03429, and the Life Insurance Medical Research Fund. We thank C. Y. Omoto, W. Hayashi, M. P. King, M. M. Clark, and A. Tam for technical assistance.

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Functional Ribosomal Unit of Gamma-Globulin Synthesis

Abstract. *Nascent protein synthesis is associated with polyribosomes in extracts of lymph node cells removed from hyperimmunized rabbits, if the ribonuclease activity in such extracts is inhibited. Prior treatment of the cells with actinomycin D markedly decreases protein synthesis by polyribosomes.*

A unique characteristic of antibody formation is that an individual animal, after appropriate stimulation with an antigen, can produce antibody molecules of a seemingly unlimited number of different specificities. The possibility has been considered, therefore, that the mechanism of γ-globulin synthesis differs from that of other mammalian proteins.

In the mammalian systems that have been examined under conditions which preserve ribosomal aggregates, protein synthesis occurs on clusters of ribosomes (polyribosomes) held together by a messenger RNA (mRNA) molecule (*1*, *2*). The average number of ribosomes forming a single polyribosome is approximately proportional to the size of the mRNA (*3*). Further, polyribosome-mediated protein synthesis slowly ceases when the synthesis of new RNA is prevented with actinomycin D (*2*).

The synthesis of antibody can be inhibited by actinomycin D (*4*); this suggests but does not prove (*5*) that γ-globulin synthesis is also dependent upon the continued synthesis of mRNA. Stenzel *et al.* (*6*) have studied the functional ribosomal unit of protein synthesis in spleen cells from immunized rabbits and have suggested that the polypeptides of γ-globulin are synthesized on single ribosomes and dimers, and thus the synthesis of γ-globulin differs from that of other mammalian proteins which have been investigated. In our experiments we show that protein synthesis by lymph node cells obtained from hyperimmune rabbits occurs on polyribosomes and that treatment of such cells in vitro with actinomycin D inhibits protein synthesis.

Rabbits were hyperimmunized with T2 phage and, 4 to 9 days after the last immunizing injection, cells were "teased" from the popliteal lymph nodes, washed, and resuspended (2 to 4×10^7 cells/ml) in either tris-buffered 1066 medium (*7*) supplemented with fetal-calf serum (20 percent) or Eagle's medium (*8*) containing 1/10 to 1/50 the standard concentrations of amino acids. Cells were stirred gently for 1 hour at 37°C and then exposed to 10 μc of C^{14}-labeled amino acids (*9*) for 1.5 to 3 minutes in order to label only nascent protein (*1*, *2*). The cells were washed three times in chilled Earle's saline solution, and 4×10^7 cells were resuspended in 1 ml of cold hypotonic buffer ($10^{-2}M$ KCl; $10^{-2}M$ tris-HCl, *p*H 7.4; $1.5 \times 10^{-3}M$ $MgCl_2$).

In the initial experiments, "pulse"-labeled lymphocytes were disrupted in a number of ways: (i) with a Dounce homogenizer after suspension of the cells in hypotonic buffer alone, or in buffer supplemented with either 10^{-4} mg of hydrocortisone per milliliter or 0.1 percent Tween 80 (*10*) (these additions increased the cell breakage); (ii) with a Potter-Elvehjem homogenizer after suspension of cells in either hypotonic buffer or the same buffer supplemented with $0.25M$ sucrose; (iii) with 0.5 percent deoxycholate.

The cultivation of S3 HeLa cells, the preparation of cytoplasmic extracts, the determination of ultraviolet absorbancy at 260 mμ, and the determination of the radioactivity insoluble in trichloroacetic acid (TCA) have already been described (*11*).

When extracts from labeled lymphocytes were prepared as described, almost all of the radioactivity was associated with either single ribosomes or small aggregates of two to three ribosomes. Since a minute amount of pancreatic ribonuclease causes similar breakdown of HeLa cell polyribosomes under the same conditions (*2*), experiments were undertaken to determine whether this association of nascent protein with single ribosomes was due to breakdown of polyribosomes. Lymphocyte extracts were mixed with HeLa cell cytoplasm which had been treated with C^{14}-labeled amino acids for 1.5 minutes, and the mixture was analyzed on sucrose gradients along with untreated HeLa cell cytoplasm (Fig. 1). In the cytoplasm of the control HeLa cell the ultraviolet-absorbing material (which is mostly due to ribosomal RNA) is in both the region of the single-ribosomes (74*S*) and in the lower part (100 to 350*S*) of the sucrose gradient. The heavier material contains polyribosomes composed of 5 to 30 ribosomes each (*12*). In agreement with the results of Penman *et al.* (*2*), nascent protein (as determined by TCA-precipitable radioactivity) is primarily associated with polyribosomes, or has been released from the ribosomes, and is found at the top of the gradient. Very little radioactivity is associated with single ribosomes. When the same HeLa cell cytoplasm is mixed with lymphocyte extracts (Fig. 1), both ultraviolet absorbancy and radioactivity are lost from the polyribosome fraction and are associated with single ribosomes and small aggregates.

In the second part of this experiment extracts prepared from labeled lymphocytes were mixed with unlabeled HeLa cell cytoplasm (Fig. 2). The HeLa cell polyribosomes were broken down as before, and lymphocyte nascent proteins were again associated with single ribosomes and small aggregates (Fig. 2). This was true of lymphocyte extracts

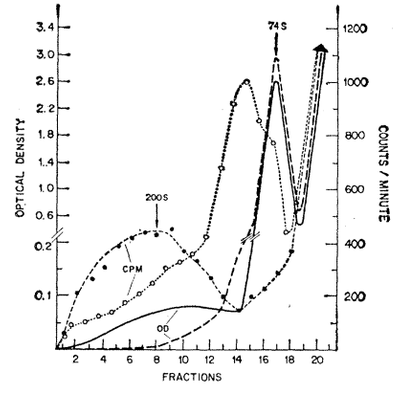

Fig. 1. Breakdown of HeLa cell polyribosomes by lymph node cell extracts. HeLa cells (8×10^7) were treated (pulse) with C^{14}-labeled amino acids, and cytoplasm was prepared and divided into two portions. One portion was mixed with the lysate from 4×10^7 lymph node cells, and both the treated sample and the untreated control were layered on a sucrose gradient (15 to 30 percent) and were centrifuged for 120 minutes at 24,000 rev/min in a SW 25.1 swinging-bucket rotor. The bottom of the gradient is on the left and the top is on the right. ——, $O.D._{260}$ of untreated HeLa cell cytoplasm; ●----●, TCA-precipitable radioactive fraction from untreated HeLa cell cytoplasm; – – – –, $O.D._{260}$ of lymph node cell plus treated HeLa cell cytoplasm; ○···○, TCA-precipitable radioactive fraction of treated HeLa cell cytoplasm.