for the different sea-urchin species should not be considered merely as intermediate decomposition products of the yolk proteins, but as active components in the cytoplasm of the developing egg.

This study was carried out at the Zoological Station of Naples, Italy in cooperation with Prof. Dr. F. BALTZER, to whom I should like to express my sincere thanks for taking care of the materials and counting the samples, and for his valuable discussions in the course of the experimental work. Thanks are also due to Prof. Dr. L. VON UBISCH for supplying a part of the *Echinocardium* eggs investigated by us. The investigation was aided by a research grant from the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung*. Finally I should like to thank the authorities at the Zoological Station of Naples for providing the working facilities.

P. S. CHEN

Institute of Zoology and Comparative Anatomy, University of Zurich, July 1, 1958.

Zusammenfassung

Als Fortsetzung früherer Untersuchungen der freien Aminosäuren und Peptiden in Eiern und Embryonen verschiedener Seeigelarten, wurde das Muster der Ninhydrin-positiven Stoffe von *Echinocardium cordatum*, *Psammechinus microtuberculatus* und *Genocidaris maculata* papierchromatographisch untersucht. Die *Echinocardium*-Eier zeichnen sich durch ihre hohe Konzentration an Valin (Fig. 1*A*, Fleck 16) und Leucin (Fleck 9) aus, welche bei allen übrigen von uns untersuchten Arten nur in sehr geringer Menge auftreten. Ferner sind sie durch das Vorkommen eines spezifischen Stoffes charakterisiert, der nur bei *Arbacia lixula* nachgewiesen wurde (Fleck 17). *Psammechinus microtuberculatus* und *Genocidaris maculata* unterscheiden sich in den Peptiden. Die *Psammechinus*-Eier enthalten einen Stoff, der wahrscheinlich mit dem Tripeptid von *Sphaerechinus granularis* identisch ist (Fig. 1*B*, Fleck Nr. 23). In *Genocidaris*-Eiern wurde ein Peptid registriert, das auch bei *Paracentrotus lividus* vorkommt (Fig. 1*C*, Fleck Nr. 21).

Es wurden ferner Messungen von Eivolumen, Totalstickstoff und Totalmenge der freien Ninhydrin-positiven Substanzen des unbefruchteten Eies durchgeführt. Das Eivolumen und der Gesamtstickstoffgehalt sind bei *Echinocardium cordatum* am grössten, bei *Arbacia lixula* am geringsten. Die *Echinocardium*-Eier sind durch ihren hohen Gehalt an freien Aminosäuren gekennzeichnet. Sie enthalten rund 7mal mehr solche Stoffe pro Ei als die *Arbacia*-Eier; zwischen *Paracentrotus lividus* und *Sphaerechinus granularis* zeigen die Messwerte keinen deutlichen Unterschied.

Fat Formation and Glycolysis in Tissue Culture Action of Hydrocortisone[1]

The question of the role of adreno-cortical steroids in fat metabolism remains as yet unsettled. On the one hand it appears that hydrocortisone is the only physiological compound that can cause obesity when administered in excess of body needs and that this type of obesity is indistinguishable from that of Cushing's syndrom. A synergistic effect of insulin and cortisone in increasing fat synthesis from carbohydrate was reported[2]. But on the other hand, antagonistic effects of cortisone and insulin on fatty acid synthesis from labeled acetate was described, cortisone having an inhibitory effect[2]. It has therefore been thought that information gained from a study of the direct action of hydrocortisone on fat formation in tissue culture may shed some light on this question. Since, however, the tendency to accumulate fat is a characteristic feature of tissue culture cells in general, even of such cell types which in the organism do not show a tendency to fat accumulation, a preliminary study of fat formation in normal untreated tissue culture cells appeared necessary.

Now, for many years, 2 distinctive characteristics of cultured cells have been observed. First, the presence of more or less high glycolysis[3], even in the presence of oxygen[4], and, second, a striking tendency to fat accumulation in cytoplasmic vacuoles[5]. It is tempting to assume that these 2 special characteristics of cultured cells are not independent of each other. From investigation into the action of high glucose concentration on respiration and glycolysis on the one hand, and on formation of fat vacuoles on the other hand, a possible relationship between glycolysis and lipogenesis has emerged, and the assumption has been made that glycolysis as a reducing system favors fatty acid synthesis. Lipogenesis requires a reducing system for the regeneration of reduced pyridine nucleotide necessary for fatty acid synthesis.

Methods.– For observation of lipid granules, the hanging drop method and a medium consisting of plasma and chick amniotic fluid[6] was used. Control cultures in amniotic fluid show far less fat accumulation than in other culture media[6]. In the first 2–4 days of growth, primary cultures of various tissues in a medium of plasma and amniotic fluid showed less fat accumulation, as estimated by number and size of fat vacuoles observed with 120 × magnification, than cultures in a medium containing embryo extract as well. If the media were not changed for several days, fat vacuoles gradually increased in number and size; by renewal of medium, or aeration, fat vacuoles could partly be removed. Mitoses were observed in cells containing large fat vacuoles. The lipid appeared predominantly to be produced by the cell and not to come from the medium, because it could be removed or reduced by renewal of the medium. Furthermore, cells in pure saline with glucose and with explants removed also showed fat vacuoles. Lipid containing cells often continued to produce acid mucopolysaccharide, as revealed by the method of mucin clot formation[7]. Addition of 0·5 to 2% glucose to the medium resulted in considerable increase of fat accumulation in the cytoplasm, compared with cultures growing in a medium of plasma and amniotic fluid only.

For investigation of the action of higher glucose concentrations on respiration and glycolysis, strain L derived from a fibroblast of mouse subcutaneous tissue was used

[1] Reported in part at 8th Annual Meeting of Tissue Culture Association at the University of Baltimore, Md., April 17, 1957.

[2] V. A. NAJJAR, *Symposium on Fat Metabolism* (The Johns Hopkins Press, Baltimore 1954).

[3] F. WIND, Biochem. Z. *179*, 384 (1926). – O. WARBURG and F. KUBOWITZ, Biochem. Z. *189*, 242 (1927). – F. LIPMANN, Biochem. Z. *261*, 157 (1933). – H. LASER, Biochem. Z. *264*, 72 (1933). – M. JONES and S. L. BONTING, Exp. Cell Res. *10*, 631 (1956). – H. HARRIS, Brit. J. exp. Path. *37*, 512 (1956). – J. PAUL and E. S. PEARSON, Exp. Cell Res. *12*, 212, 223 (1957). – H. GROSSFELD, Science *127*, 148 (1958).

[4] H. LASER, Biochem. Z. *264*, 72 (1933). – H. HARRIS, Brit. J. exp. Path. *37*, 512 (1956). – H. GROSSFELD, Science *127*, 148 (1958).

[5] A. A. KRONTOWSKI, Arch. exp. Zellforsch. *11*, 94 (1931). – L. DOLJANSKI, Arch. exp. Zellforsch. *11*, 261 (1931). – D. IGNATOWITCH, C. R. Soc. Biol. *76*, 607 (1914). – J. ZWEIBAUM, Arch. exp. Zellforsch. *15*, 391 (1934). – R. A. LAMBERT and F. M. HANES, Virchows Arch. *211*, 116 (1913). – J. J. BIESELE and P. GOLDHABER, Cancer Res. *15*, 767 (1955).

[6] H. GROSSFELD, Proc. Soc. exp. Biol. Med. *71*, 475 (1949).

[7] H. GROSSFELD, Exp. Cell Res. *14*, 213 (1958).

and the following procedure was employed. Cells from 4 T–60 flasks, each containing about 15×10^6 cells, were scraped from the glass, pooled, centrifuged and the residue suspended in 12 ml fresh medium (with phenol red as pH indicator, pH 7·4), consisting of embryo extract, horse serum and Earle's balanced salt solution (containing 0·1% glucose and 0·22% $NaHCO_3$), in proportions of 20 : 40 : 40. 3 ml of the cell suspension were put into each of 4 Warburg flasks. To 2 of the Warburg flasks, 0·5 ml of Earle's solution containing glucose in final concentration of 1% was added, and to 2 control flasks 0·5 ml of Earle's solution without additional glucose. One control and one experimental flask contained 0·2 ml of 15% KOH in the central well for measurement of oxygen uptake; one control and one experimental flask contained 0·2 ml of H_2O in the central well. In this way, aerobic glycolysis could be determined by the difference between the readings on the flasks with and without alkali[8].

The results are shown in Figure 1. At higher glucose concentration, respiration is depressed and glycolysis increased. It appears that with excess of glucose, the cultured cell may switch to glycolysis, the less complex and less economic source of energy. The present results are in agreement with those obtained by JONES and BONTING[9] by lactate determination.

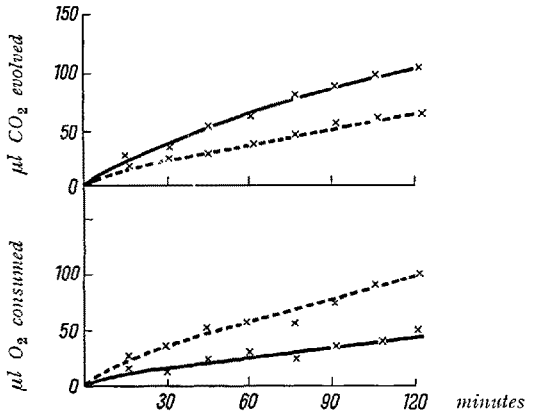

Fig. 1. ——— 1·1 % glucose - - - 0·1 % glucose Strain L

Increase of cytoplasmic lipid vacuoles in cells treated with hydrocortisone in high concentration was reported[10]. It was therefore of interest to test the effect of this glucocorticoid on respiration and glycolysis and the above described procedure of measuring respiration and aerobic glycolysis has been employed with strain L cells treated with hydrocortisone in concentration of 125 mg/ml. The results are shown in Figure 2 which demonstrates a stimulation of aerobic glycolysis and depression of respiration by concentrations known to increase fat vacuole formation. Glycolysis as a reducing system (and maybe also depression of respiration) appears to be involved in increased fat accumulation.

Discussion. Increased glycolysis in tissue culture had been found first by WIND[11] and by WARBURG and KUBOWITZ[12] ($Q^{N_2}_{CO_2}$, was 40 to 50, against only 15 in the embryo from which the explanted tissue derived). This was confirmed by a great number of investigators[13], and many of them found also aerobic glycolysis in tissue culture, although the ratio of aerobic glycolysis to respiration seemed to vary under various conditions. HARRIS[14] recently reported that under both aerobic and anaerobic conditions most of the glucose consumed by the cells in tissue culture was converted to lactic acid. It is also evident that the unusually high consumption of glucose by cultured cells[15] is a result of prevailing glycolysis which is an uneconomical way of glucose utilization.

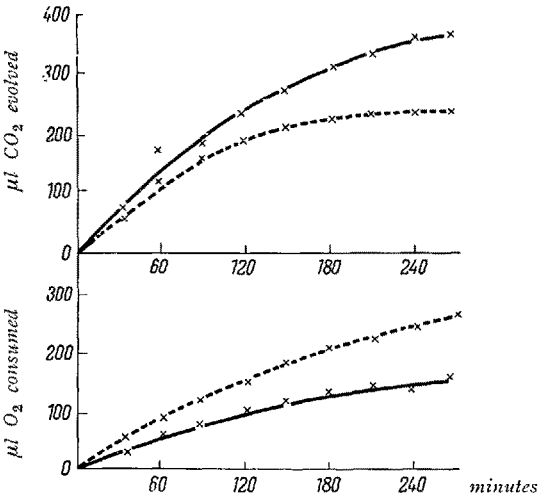

Fig. 2. ——— HC - - - control Strain L

The link between glucose concentration and glycolysis on the one hand and lipogenesis on the other hand appeared to be the fact that fatty acid synthesis requires a reducing system for regeneration of DPNH. Glycolysis providing a reductive environment with electron donors for regeneration of DPNH may be under certain conditions the driving force for reversing the oxidative pathway in the direction of lipogenesis[16]. Fatty acid synthesis is accomplished through repetition of a cycle of reactions, with condensation of two molecules of acetyl CoA, and reduction of acetoacetyl CoA to β-hydroxybutyryl CoA. In the presence of β-keto reductase, acetyl-S-CoA is reduced by DPNH. The reaction products are DPN, β-hydroxybutyryl-S-CoA and HS-CoA. This is followed by dehydration to crotonyl CoA, and its reduction to butyryl CoA. A new cycle is started by the reaction of butyryl CoA with another molecule of acetyl CoA, to form β-keto-caproyl CoA + CoA-SH, and so forth. The cycle is repeated eight times until stearyl CoA is formed[17]. Fatty acid synthesis requires thus the participation of reduced pyridine nucleotide. Since the oxidation of lactate is coupled with the reduction of DPN, the ability of lactate to regenerate reduced pyridyne nucleotide may be a key factor in stimulating lipogenesis[16]. Furthermore, the

[8] H. GROSSFELD, Science *127*, 148 (1958).

[9] M. JONES and S. L. BONTING, Exp. Cell Res. *10*, 631 (1956).

[10] H. GROSSFELD and CH. RAGAN, Proc. Soc. exp. Biol. Med. *86*, 63 (1954). – A. S. STEEN, Brit. J. Ophth. *35*, 741 (1951). – M. HOLDEN and L. B. ADAMS, Proc. Soc. exp. Biol. Med. *95*, 364 (1957). – G. LENTI, E. TORTAROLO, G. MASOLINO, and D. TAPPERO, Folia endocrin. *4*, 743 (1957).

[11] F. WIND, Biochem. Z. *179*, 384 (1926).

[12] O. WARBURG and F. KUBOWITZ, Biochem. Z. *189*, 242 (1927).

[13] F. LIPMANN, Biochem. Z. *261*, 157 (1933). – H. LASER, Biochem. Z. *264*, 72 (1933). – M. JONES and S. L. BONTING, Exp. Cell Res. *10*, 631 (1956). – H. HARRIS, Brit. J. exp. Path. *37*, 512 (1956). – J. PAUL and E. S. PEARSON, Exp. Cell Res. *12*, 212, 223 (1957). – H. GROSSFELD, Science *127*, 148 (1958).

[14] H. HARRIS, Brit. J. exp. Path. *37*, 512 (1956).

[15] S. GRAFF and K. S. MCCARTY, Exp. Cell Res. *13*, 348 (1957).

[16] H. GROSSFELD, *Eighth Annual Meeting of the Tissue Culture Association Baltimore*, April 1957 (Abstr.). – CH. TERNER, Proc. Soc. exp. Biol. Med. *96*, 801 (1957).

[17] F. LYNEN, Harvey Lectures *48*, 212 (1954).

ability of glucose to regenerate reduced pyridine nucleotide during oxidation of phosphoglyceraldehyde to phosphoglyceric acid, where the hydrogen transfer likewise proceeds by way of diphosphopyridine nucleotide, may explain its effectiveness in stimulating fatty acid synthesis.

It may be worthwile mentioning that as end products of fermentation in worms, which consume six times as much glycogen in fermentation than in respiration, in addition to lactic acid, large amounts of higher fatty acids have been found[18]. It is also tempting to correlate the striking accumulation of lipid in the cytoplasm of healthy growing chondrocytes with the high glycolysis present in cartilage. In conclusion, a reducing system appears to be essential for efficient lipogenesis. Excess glucose increases both glycolysis and fat accumulation in cultured cells. Augmentation of lipid vacuoles observed in cultures treated with high concentrations of hydrocortisone appears to be connected with increase of glycolysis (and depression of respiration) by hydrocortisone in high concentration.

H. GROSSFELD*

Departments of Medicine and Orthopedic Surgery, Columbia University, College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, New York City, April 25, 1958.

Zusammenfassung

Fettsäuresynthese wird durch die Anwesenheit eines glykolytischen reduzierenden Systems gefördert. Die auffallende Tendenz normaler Gewebskulturzellen zur Fettanhäufung im Zytoplasma scheint mit dem charakteristisch hohen Fermentationsstoffwechsel der Gewebskulturen zusammenzuhängen. Die Steigerung der Fettbildung durch Hydrocortison in hoher Konzentration mag auch in Zusammenhang stehen mit dessen Eigenschaft, Spaltungsstoffwechsel zu fördern.

[18] W. R. SLATER, Biochem. J. *19*, 604 (1926). – T. v. BRAND, Erg. Biol. *10*, 37 (1934).

* Supported by U. S. Public Health Service grant.

Effects of Cortisone and Ethylenediamine-Tetraacetic Acid on Deposition of Promethium (Pm^{147})

In this investigation cortisone, which has been reported to affect bone metabolism[1], was administered with the calcium salt of ethylenediamine-tetraacetic acid (CaEDTA) to enhance its effects on retention of promethium (Pm^{147}) in skeleton and other tissues of rats.

Twelve and one-half μc of $Pm^{147}Cl_3$ (half-life, 2·6 years) in 0·25 ml of dilute NaCl solution, pH 3, were injected intravenously into 20 young female Wistar rats (wt. range, 160–185 g). Of these rats three groups of five animals each were subsequently treated with CaEDTA and/or cortisone acetate according to the schema shown in Table I. Five rats received no treatment and served as controls.

The animals were maintained *ad libitum* on a standard stock diet throughout the 72-hour experimental period. Liver, spleen, kidney, and one femur from the killed animals were muffled and the residual ash dissolved in N/10 HCl. Samples of whole plasma, and of plasma proteins obtained by alcohol precipitation, were taken from the animals. An undecalcified femur from each animal was longitudinally transected and prepared for contact autoradiography. The sections were exposed for 72 hours at 4° C on No-screen x-ray film.

Concentrations of Pm^{147} in aliquots of the samples were measured in a gas counter. After correcting for self-absorption the radioactivity present in the samples was compared to aliquots of the original injection solution.

Tissue Distribution. The concentrations of Pm^{147} in the samples were found to be independent of the organs' weights. These data are expressed as per cent of administered dose per organ in Table II. This table also presents Pm^{147} concentrations in plasma and plasma proteins.

In each of the animal groups the liver contained the largest fractions of the injected Pm^{147}, in agreement with an early study[2]. As in a recent study[3] the amount of Pm^{147} in liver was decreased by 20% by CaEDTA treatment, a difference which was statistically valid at the 99% level of significance. No effects of CaEDTA treatment on retention of Pm^{147} in spleen, kidney or femur were detected.

In contrast to the marked localization of Pm^{147} in liver only small amounts were found in spleen. This suggests correspondingly that there was little uptake of radiocolloidal Pm^{147} by the reticuloendothelial components of the spleen. By inference much of the injected and circulating Pm^{147} may have been relatively diffusible.

The table shows that, in contrast to its depressant action on early uptake by spleen and enhancement of femur uptake of radioyttrium[4], cortisone treatment had no effect on Pm^{147} uptake by these tissues. However, these

[1] R. H. FOLLIS, JR., Proc. Soc. exp. Biol. Med. *76*, 722 (1951).

[2] J. G. HAMILTON, Rev. Mod. Phys. *20*, 718 (1948).

[3] H. FOREMAN and C. FINNEGAN, J. Biol. Chem. *226*, 745 (1957).

[4] B. KAWIN, Nature *179*, 871 (1957).

Table I

Sequence of treatments following intravenous Pm^{147} injection [a]

Treatment group \ Time	1	2	21	22	45	46	69	70	72
	Hours after Pm^{147} injection								
Controls	– No treatments –								Sacrifice
Cortisone[b]	Cortisone		Cortisone		Cortisone		Cortisone		Sacrifice
CaEDTA[c]		CaEDTA		CaEDTA		CaEDTA		CaEDTA	Sacrifice
Cortisone-CaEDTA	Cortisone	CaEDTA	Cortisone	CaEDTA	Cortisone	CaEDTA	Cortisone	CaEDTA	Sacrifice

[a] $Pm^{147}Cl_3$, carrier-free, half-life 2·6 years. Dose: 12·5 μc/0·25 ml/animal, pH 3, in dilute NaCl.

[b] Cortisone acetate. Dose: 50 mg/kg body weight, as 'Cortone' (Merck), 25 gm/ml with added suspending agents and 1·5% benzyl alcohol as preservative.

[c] Calcium salt of 'Sequesterene' (Alrose Chemical). Dose 50 mg/0·5 ml/animal, pH 7·1.