in the course of the first minutes and then to remain almost constant. We lack thus any indication of the presence of rapidly renewed fatty acid fraction in the brain. The specific activity values of the muscle fatty acids extracted from different groups of mice fluctuate considerably. An indication of a decrease of the specific activity figures within a 240 min. observation period is, however, also in this case absent.

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Zusammenfassung

Wird in der Carboxylgruppe gezeichnetes Azetat Mäusen eingespritzt, so findet man, daß die spezifische Aktivität der Fettsäure der Leber schon nach 30 Minuten erheblich abgenommen hat. Dieser Befund weist darauf hin, daß die Fettsäure der Leber eine Fraktion enthält, die in bedeutend rascherem Tempo als das durchschnittliche Fettsäuremolekül jenes Organs erneuert wird. Verschiedene Forscher haben festgestellt, daß diesem eine Halblebenszeit von rund einem Tag zukommt.

Keine Fettsäurefraktion mit raschem Erneuerungstempo konnte im Hirn bzw. Muskel der Maus festgestellt werden.

On Glycolysis in the Chicken Embryo

MEYERHOF and PERDIGON[1] have established the fact that extracts of 3–9 day old chicken embryos form—in anaerobiosis—lactic acid from fructofuranose-1,6-diphosphoric acid (HDP). They show that in order to obtain the complete glycolytic activity of the extracts it is indispensable to add diphosphopyridinenucleotide (DPN) because the DPN preformed by the embryos is very quickly inactivated at 37°C. Recently NOVIKOFF, POTTER and LE PAGE[2] show that homogenates of 5–8 days old embryos glycolysate—in oxi- and anoxibiosis-HDP after the addition to the enzymatic system of DPN and ATP, and fructofuranose-6-phosphoric acid, glucopyranose-6-phosphoric acid and glucose when, in addition to DPN and ATP, HDP is present in the system. The researches of MEYERHOF and PERDIGON, and of NOVIKOFF, POTTER and LE PAGE thus show fully the existence of a glycolytic system in chicken embryo, which action develops through the formation and breakdown of phosphoric esters, in accordance with EMBDEN-MEYERHOF'S cycle.

The importance of these results is easily appreciated if one thinks of the general opinion particularly based on the researchs of NEEDHAM and coll.[3], that anaerobic glycolysis would have in the embryo a course independent of the formation of phosphoric esters. A non-phosphorylating glycolysis seems really to exist in the brain[1] and in various kinds of Fusarium[2], where it follows a completely different route from the one observed in muscle and yeast. Even though NOVIKOFF, POTTER and LE PAGE, as well as previously DORFMAN[3], write: "Though it does not rule out the possibility of a non-phosphorylating route of glycolysis, the available evidence does not make necessary postulating the existence of such a route", two experiments performed by them may cause one to doubt–as they themselves admit—that the phosphorylating glycolysis is the only route open for the catabolism of glycides in the embryo. In one, in fact, the homogenate of fresh embryo does not show the typical need of HDP for glycolysis of the glucose, in the other neither the DPN nor the ATP omission provoke appreciable changes in the reaction. Therefore we thought it was interesting to show the existence in chicken embryos of a non-phosphorylating breakdown of the glycides and the separation of the enzymatic system provoking it from the one provoking the glycolysis preceded by the phosphorylation. By repeated extractions with Ringer solution of 7–8 days old chicken embryo we could easily remove from the tissue the enzymes catalysing the process of phosphorylating glycolysis. On the contrary another enzymatic system, easily forming lactic acid from glucose (Table I) resisted the extraction.

Table I

Formation of lactic acid from glucose. Reaction mixture: 1 g embryo after the extraction; 5 ml Ringer's-bicarbonate solution; 25 mg glucose.

Added substances	mg of total lactic acid		Mean mg	mg of formed lactic acid
	Exp. I	Exp. II		
—	0·22	0·24	0·23	–
Glucose. . . .	2·94	2·94	2·94	2·71

Moreover the formation of lactic acid from glucose is not modified by the presence of DPN, ATP and HDP (table II).

Table II

Action of DPN, ATP, HDP on the formation of lactic acid from glucose. Reaction mixture: 1 g embryo after the extraction; 5 ml Ringer's-bicarbonate solution; 0·25 ml, 0·1 mol phosphates; 25 mg glucose.

Added substances	mg of total lactic acid		Mean mg	mg of formed lactic acid
	Exp. I	Exp. II		
—	0·27	0·28	0·27	–
Glucose. . . .	2·04	2·01	2·02	1·75
Glucose + 2·5 mg DPN + 4 mg ATP + 0·1 mg HDP	2·06	1·94	2·00	1·73

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Also adenosintriphosphatase (ATP-ase) has no importance for the glycolysis of the extracted embryonic tissue. Though most of this enzymes, as MEYERHOF and WILSON affirmed[1], is transferred to the solution used for the extraction, a part of it remains in the tissue and, in contrast to the one transferred to the extract[2], is strongly inhibited by toluene, sodium azide, digitonin, not so strongly by octyl alcohol (Table III), thus remaining an analogous inhibition of the enzyme of homogenates of tumoral tissue (MEYERHOF and WILSON)[3].

Table III

Inhibition of the ATP-ase of the embryo with toluene, octyl alcohol, sodium azide, and digitonin, after the extraction. Reaction mixture: 50 mg embryonic tissue after the extraction; 0·6 ml, 0·5 mol diethylbarbiturate p_H 7·4; 0·2 ml, 0·04 mol $CaCl_2$; 0·6 ml, 0·013 mol ATP p_H 7·4 and 0·8 ml H_2O.

Inhibitory substances	Concentration	Inorganic P splitt off γ	Inhibition %
—	—	39·4	—
Toluene	saturated	9·4	76
Octyl alcohol . . .	saturated	26·2	33
Natrium azide . . .	0·2 %	16·9	57
Digitonin	0·12%	16·9	57

The ATP-ase inhibition provoked by the above mentioned narcotica does not provoke glycolysis. On the contrary, as one of us could previously observe (CAFIERO[4]) in the brain, a complete abolition of the glycolysis is induced by toluene and octyl alcohol, partial abolition by digitonin; sodium azide has no action.

Similar to ATP-ase, the pherase hexokinase seems to be important for a satisfactory development of the phosphorylating glycolysis in the tissue homogenates[5]. Addition of hexokinase to the experimental system employed both in presence and absence of DPN, ATP, HDP, does not modify at all the speed of the process.

Therefore it appears that 7–8 days old chicken embryos, after repeated extraction with Ringer's solution, form lactic acid from glucose, following a route which is different from the one of the phosphorylation cycle or for which, at least, it is impossible to acknowledge the reactions of that cycle. As possibly the non-phosphorylating glycolysis of the extracted embryonic tissue depends only on the difficulty of equilibrium among the cellular enzymes provoked by the extraction, we cannot affirm that in the healthy embryo a non-phosphorylating route of the glycolysis is accompanied by the phosphorylating one.

Experimental.–7–8 day old embryos, obtained by the incubation of eggs of Leghorn hens in an electric incubator, were quickly isolated from their annexes and reduced in an fine extract in a mortar to 0°C. Shortly afterward an extract was obtained with the addition of 5 parts of Ringer's solution cooled to 0°C, stirring continually for 20 min., each time then centrifuging and decanting the solution. Equal amounts of the extracts were quickly weighed and placed in large conical Barcroft-Warburg flask, supplied with a lateral diverticulum. 5 ml of Ringer's solution with the addition of 0·03 mol $NaHCO_3$ and eventually of the testing substrates were added. The reaction mixtures were saturated during 15 min. with a N_2 current, containing 5% CO_2 and put in a thermostat at 37°C during 3 hours. The p_H of the reaction mixture was 7·4–7·5. The determinations of lactic acid were obtained using method and apparatus of LIEB and ZACHERL[1] after deproteinisation of the samples according to FOLIN and WU and after removal of glycides by the cuprocalcium precipitation. The activity of the ATP-ase was determined according to DU BOIS and POTTER[2].

DPN was a product of Nutritional Biochem. Co. and ATP of Sigma Chem. Co. Hexokinase was prepared according to MEYERHOF's method[3].

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Zusammenfassung

In mit Ringerlösung erhaltenen Extrakten aus 7–8 Tage alten Hühnerembryonen bildet sich anaerobiotisch aus Glukose Milchsäure, und zwar gleichgültig ob DPN, ATP oder HDP vorhanden ist oder nicht. Diese Reaktion wird durch Toluol und Oktylalkohol vollständig und durch Digitonin teilweise gehemmt. Natriumazid ist dagegen ohne Wirkung. Beigefügte Hexokinase beeinflußt den Vorgang nicht. Die ATP-ase des extrahierten embryonalen Gewebes wird durch Toluol, Oktylalkohol, Natriumazid und Digitonin deutlich gehemmt.

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Foamproduction of Bloodserum and the Relation to its Proteincontent

Bloodserum as a colloidal proteinsolution produces foam under certain conditions. The amount of foam depends on the amount of serum applied. However, by using equal quantities of serum, foamproduction is in direct relation to the protein content of the blood. In order to work with a standardized but simple method, the following procedure proved to be useful: 0·2 serum diluted with 5 cm³ *aqua destillata* are vigorously shaken during 20 seconds in a test-tube of 15 cm height and 1·2 cm width. The size or height of the foam column is measured after 10 and 20 minutes. The total-protein content of the serum was determined by the methods of CONVAY and of KJELDHAL. The results are illustrated by figure 1. The method allows only a rough estimation which, for practical purposes proved to be sufficient. A normal total-protein content of the blood between 6·5 and 8·0 g% corresponds to a foam column of 2·2 to 2·8 cm height, after 10 minutes (in figure 1). A hypoproteinemia of 4% or less produces a foam column of about 1·5 cm. (I, a case of portal hypertension with total-protein 3·6 g%.) A hyperproteinemia of 9 or 11 or more g% builds a foam of 3·5 to 4·0 cm. (III, a case of multiple myeloma, total-protein 12 g%.)

In order to determine the role which the three different protein constituents play in foam production, experiments were carried out with fibrinogen, with globulins,

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