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.

[1] H. LIEB and M. K. ZACHERL, Z. physiol. Chem. *211*, 211 (1932).
[2] K. P. DU BOIS and V. R. POTTER, J. Biol. Chem. *150*, 185 (1943).
[3] O. MEYERHOF, Biochem. Z. *183*, 176 (1927).

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 cm^3 *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,

[1] O. MEYERHOF and J. R. WILSON, Arch. Biochem. *23*, 246 (1949).
[2] O. MEYERHOF and J. R. WILSON, Arch. Biochem. *21*, 1 (1949).
[3] H. LIEB and M. K. ZACHERL, Z. physiol. Chem. *211*, 211 (1932).
[4] F. P. MAZZA and C. LENTI, Arch. Sc. Biol. *24*, 203 (1938); *25*, 447 (1939); *28*, 245 (1942). – C. LENTI and M. FUORTES, Atti Acc. Sci. Torino *73*, 228 (1937–1938). – C. LENTI and N. BARGONI, Arch. Med. sper. *15*, 71 (1944). – C. LENTI, Boll. Soc. ital. Biol. sper. *20*, 530 (1945); Rend. Acc. Naz. Lincei *5*, 519 (1948). – M. CAFIERO, Boll. Soc. ital. Biol. sper. *25*, 1265 (1949).
[5] O. MEYERHOF and J. R. WILSON, Arch. Biochem. *23*, 246 (1949); *21*, 1 (1949).

with albumin and with mixtures of the three. Fibrinogen hardly produces any foam. Globulins build a good foam column but they do not reach the albumin results. Albumin is the main factor in foam building. Mixtures of the albumin and globulin yield more foam than the column equivalents of each of these proteins separately. Since fibrinogen is of no importance, the test can and should be made with serum and not with plasma.

Two more facts could be observed: (1) Quick and short heating to 100 °C before shaking increases the foam-production double or even threefold. The test-tubes are photographed by pairs, those tubes with the higher foam level demonstrating the heating effect.

(2) Though the foam column shrinks to a very small ring after standing for about two hours, the phenomenon of foam production can be quantitatively reproduced by renewed shaking and it can be repeated several times.

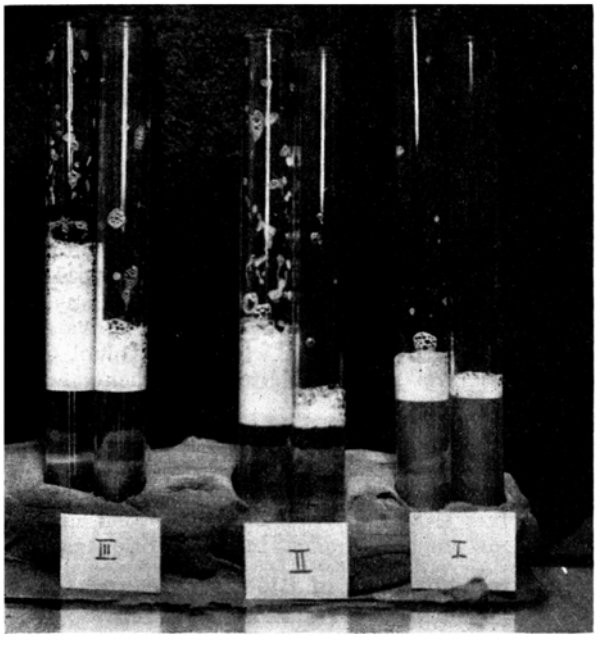

Fig. 1.

There is one exception to the rule of the relation between foam amount and the quantity of serum total-protein. It was to be expected that large amounts of lipids as highly surface-tension-active material diminish foam production. Values above 500 mg% cholesterol in the serum make the size of the foam column smaller than the actual amount of serum total-protein would yield. Serum of a diabetic patient was shaken in the described manner, before and after a fat meal. The foam column of the second specimen was about 0·5 cm less high due to the presence of 550 mg% total cholesterol Serum of a patient with a nephrotic syndrom, where the total cholesterol of the serum was 1000 mg% and total-protein 6·0 g%, had a foam column of only 1·0 cm and not 2·0 or 2·2 cm as it should be expected at this protein level.

Thus, actual lipemia or very high cholesterinemia forbid the application of this test.

Otherwise this foam test seems suitable as a quick estimation method (not determination) for total-protein in the serum.

Details of the technique and its results will be published.

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Zusammenfassung

Wenn Blutserum als eine kolloidale Eiweißlösung mit *Aqua dest.* kräftig geschüttelt wird, dann bildet sich Schaum. Der Schaum wird hauptsächlich vom Albumin gebildet. Es besteht in grober Annäherung eine direkte Beziehung zwischen Eiweißmengen und Schaumhöhe. Deshalb kann man diese einfache Methode zur raschen Schätzung des Gesamteiweißes im Serum benutzen.

Comportamento dell'azoto non proteico (A.N.P.) del sangue e dei tessuti nell'avitaminosi B_1

Generalmente non si ammettono diretti rapporti tra tiamina e metabolismo proteico e secondo qualche ricercatore risulterebbe anche dimostrato che la tiamina non deve considerarsi come un fattore essenziale per il metabolismo protidico. Infatti secondo DANN[1] ratti che ricevono una dieta sintetica contenente 80% di caseina purificata possono essere mantenuti per oltre un anno senza tiamina. Tuttavia non mancano ricerche tendenti a dimostrare che la tiamina direttamente o indirettamente è connessa con il metabolismo protidico. Prima di ogni altro i risultati di LAVROFF e JARUSSOVA[2], i quali con ricerche di bilancio azotato sul piccione tenuto a digiuno proteico (regime a base di amido), osservano che mentre in assenza di tiamina il catabolismo azotato è molto intenso (bilancio azotato fortemente negativo), in presenza di tiamina invece esso è più ridotto, l'eliminazione dell'N si stabilizza e la sopravvivenza dell'animale è prolungata. Secondo i suddetti autori, la tiamina permette all'organismo di utilizzare le sostanze ternarie economizzando proteine e stabilendo il livello della distruzione proteica a un valore più basso. L'alterato metabolismo proteico, secondo queste vedute, dipenderebbe dall'impossibilità dell'organismo beriberico di utilizzare gli idrati di carbonio.

Che il bilancio azotato diventi progressivamente negativo col procedere dell'avitaminosi è un fatto assodato da tempo: solo che la maggior parte degli autori attribuisce questo comportamento allo stato d'inanizione cui vanno incontro gli animali in avitaminosi.

Da ricerche sul bilancio azotato in ratti in avitaminosi B_1 eseguite in questo Istituto da OTTELLI[3] e da DE CARO[4] risulta che il bilancio azotato può diventare negativo nell'avitaminosi B_1 anche indipendentemente dallo stato di inanizione.

Inoltre, per quanto riguarda l'escrezione urinaria azotata, è importante considerare, in relazione ad una eventuale ridotta utilizzazione protidica, che il rapporto dell'N ureico all'N totale è abbassato (KRITZMANN[5]).

Un interessante gruppo di lavori di BRAUNSTEIN e KRITZMANN si riferisce alle alterazioni della transamminazione, che esprimerebbe una particolare insufficienza del metabolismo intermedio.

Questi autori osservano che nei tessuti (muscolo, fegato, cervello, rene) dei ratti in avitaminosi B_1, incubati con acido *l*-glutammico e piruvico, il trasferimento del

[1] W. G. DANN, Federation Proc. *4*, 153 (1945).

[2] B. A. LAVROFF e N. S. JARUSSOVA, Bull. Soc. Chim. Biol. *21*, 1139 (1939).

[3] M. OTTELLI, Quad. Nutriz. *7*, 342 (1941).

[4] L. DE CARO, Farmaco *1*, 16 (1946).

[5] H. G. KRITZMANN, Biokimyia *8*, 85 (1943), in: Brit. Abstr. [A] *3*, 422 (1944).