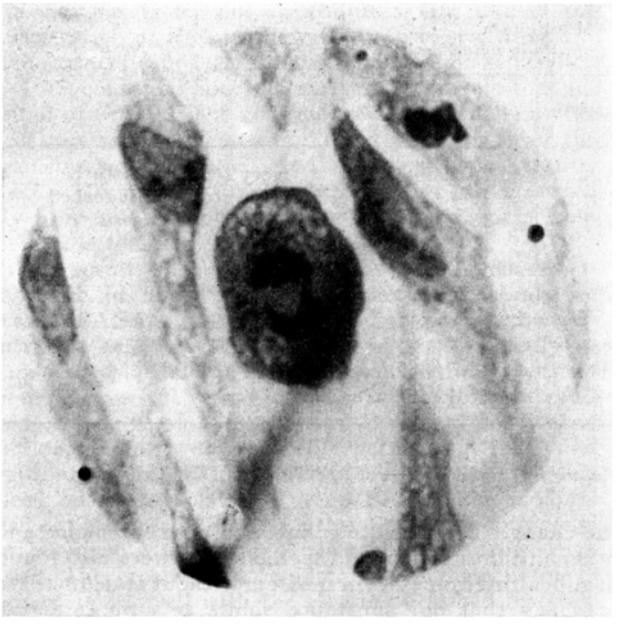

Fig. 4. – Culture of myocardial cells: after 24 h growth a drop of Italchina 1:100,000 added (M. II). Anaphase with bridge formation. Enlarged 1350 ×.

that approaches the colchicine function and is in some way different from the action of trypaflavine and other acridine derivatives. Experiments on tumor growth may be suggested.

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Riassunto

Il ben noto derivato acridinico usato nella terapia antimalarica (Italchina = Atebrina) produce un gran numero di alterazioni mitotiche nelle culture *in vitro* di mioblasti di pollo, in opposizione ad altri derivati acridinici, i quali secondo dati della letteratura impediscono l'inizio della mitosi senza disturbare poi il meccanismo mitotico. Qualche importanza nel modificare l'azione sulle cellule può forse averla la lunga catena laterale del preparato.

Turnover Rate of the Fatty Acids of the Liver

The rate of renewal of the fatty acids of the liver and other organs has been repeatedly determined by making use of deuterium, carbon 13 and carbon 14 as an indicator. In early experiments SCHOENHEIMER and RITTENBERG[1] administered heavy water to mice and kept the deuterium content of body fluids at a constant level throughout the experiment. The saturated fatty acids of the mouse reached half of their maximal deuterium content in the time of 5 to 9 days. The deuterium content of the saturated acids was higher than that of the unsaturated.

BERNHARD and SCHOENHEIMER[2] isolated the fatty acids of the intestinal wall, the kidneys and the liver of the mouse and found the saturated fatty acids to have an appreciably higher deuterium[1] content than had that of the unsaturated ones. They estimate the half life time of the average saturated fatty acid molecule in the liver of the mouse to be about 1 day, while the half life time of the total fatty acids in the rat liver was found by STETTEN and BOXER[2] to be 1·9 days.

The finding of RITTENBERG and BLOCH[3] that the feeding to mice of acetate containing C^{13} in the carboxyl group leads to the formation of fatty acids containing C^{13}, opened the way to the application of C^{13} and C^{14} in the study of the rate of formation of fatty acids. They found a more rapid incorporation of C^{13} in to the saturated than into the total fatty acids. The C^{13} concentration of the carboxyl carbon atoms of the saturated fatty acids was approximately twice as high as the average of all the carbon in the saturated fatty acids. The most plausible distribution which will explain these data is one in which the labelled carbon is present at every other carbon atom; i. e., at the odd number carbon atoms of the fatty acids. Later work[4] showed that at least 25 percent of the carbon atoms of the fatty acids are derived from acetate. Evidence wasalso obtained that acetic acid can furnish every carbon atoms of the molecule.

Recently the rate of turnover of fatty acids has been reinvestigated with the aid of acetic acid labeled by C^{14} in the carboxyl group by Pihl et al.[5]. The percentage renewal of the fatty acid molecules is determined by comparing the C^{14} content of the fatty acid carbon at the end of the experiment with the average value of the C^{14} content of the precursor carbon which prevailed during the experiment. To arrive at the last mentioned data, phenyl-DL-aminobutyric acid was fed simultaneously with labeled acetate to adult rats kept on fat free diet, and consecutive samples of the excreted acetyl derivatives were analyzed[6]. Though the labeled acetate content of the diet was kept constant, the isotope concentration of the acetyl group was found to increase in the course of the 30 days period with about 30% of the initial value. This increase was shown to be due to the catabolism of the labeled higher fatty acids formed during the experiment. The metabolic products of the labeled fatty acids contribute in these long-time experiments significant quantities of labeled acetyl groups to the acetic acid pool which supplies C^{14} to the newly formed fatty acid molecules.

The saturated fatty acids of liver were found to reach half of their maximal isotope concentration in less than 1 day, the unsaturated acids in about 2 days. Much longer time is necessary to reach a corresponding C^{14} concentration in the fatty acids of the carcass, 16–17 days for saturated and 19–20 days for the unsaturated acids. This difference was also shown in recent work of POPJAK and BEECKMANS[7].

In an investigation on the effect of changes in the metabolic rate on the incorporation of C^{14} into tissue

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fractions, we determined the specific activity of the liver fatty acids of the mouse following injection of carboxyl labeled acetate in experiments of 10 to 180 min. duration. The results obtained, which are discussed in this note, suggest, the existence of a fatty acid fraction in the mouse liver of much shorter half-life time than about 1 day, which was found in the various experiments mentioned above.

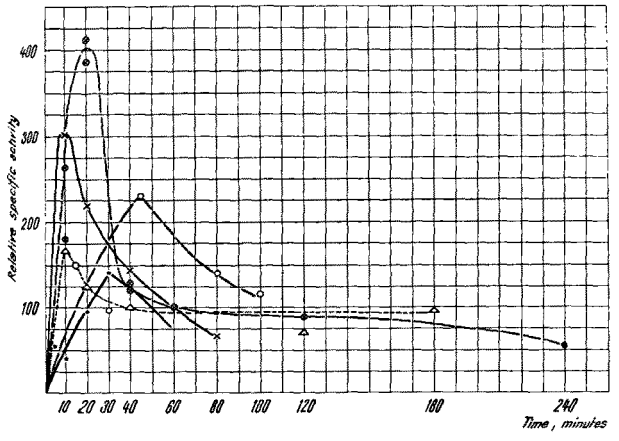

Fig. 1. – Change of the specific activity of the liver fatty acids with time

Experimental. – In each experiment 45 to 72 mice were injected intraperitoneally with 0·2 ml of 0·8% sodium chloride solution containing 2–4 microcuries (0·2–0·4 mg) of sodium acetate labelled in the carboxyl group. The animals were divided in 5–6 equal groups and killed after 5 to 240 min.

The pooled organs were frozen in solid CO_1. The ground tissue was then extracted for 3 h with a boiling mixture of ether-alcohol 1:3. The filtrate obtained was evaporated *in vacuo* and the residue extracted with petroleum-ether. The residue obtained after evaporation of the petroleum-ether was saponified for 8 h with 10 ml of 40% KOH solution and 20 ml of alcohol on a boiling waterbath.

The solution was extracted three times with petroleum-ether in order to remove the insaponifiable matter. The aqueous solution was then neutralised with 40% H_2SO_4 solution and extracted three times with petroleum-ether.

The petroleum-ether solutions on evaporation gave the fatty acids. The determination of the radioactivity was carried out with a Geiger counter, 8 mg of each sample on an aluminium disk of 5 mm diameter.

Discussion. The relative specific activity of the total fatty acids extracted from the liver of mice killed at different times after intraperitoneal injection of acetate labeled in the carboxyl group is plotted in figure 1. In view of the fact that the five experiments, the results of which are recorded, were carried out with different strains of mice, we plotted the results of each experiment by taking the value obtained after 60 min. experiment to be 100. Each point indicates a value obtained by extracting the fatty acids of 9 to 12 pooled livers. The total number of mice involved in these experiments amounts to 250. 1 mg of fatty acid of the liver of a 20 g mouse contains $8{\cdot}2 \times 10^{-4}$ part of the C^{14} administered. This figure indicates the value corresponding to the highest peak of the curves.

It takes several minutes until the injected labeled acetate or its decomposition products penetrate into the liver and are incorporated into fatty acid molecules. Correspondingly, the specific activity of the fatty acids increases for the first minutes. This time was determined for the rat to be 15 min.[1]. The increase in the specific activity of the fatty acid precursors with time is soon followed by a decrease. While the endogenous inactive acetate is constantly being produced–about 1·2 mg are formed daily per 100 mg of rat tissue[2]–the injected labeled acetate is not replaced. Correspondingly, after a while fatty acid molecules will be synthesized in the liver from almost inactive precursors. The time will also arrive when the labeled fatty acid molecules present will be renewed a second time from less active precursors than the first time. Active fatty acid molecules will thus be replaced by less active ones, and the specific activity of the average fatty acid molecule shall now decrease with time. The rate of decrease will be determined by the half life time of the liver fatty acid molecules. Let us assume that after the lapse of 30 min. no further active fatty acid molecules are formed in the liver, the precursors being no longer active, and correspondingly the specific activity of the fatty acid of the liver will decrease with time according to the formula

$$S_t = S_0 e^{-\frac{\ln 2\, t}{T}}$$

where S_o denotes the specific activity after 30 min., S_t that at any later time t, while T = half life time of the fatty acid molecules in the liver.

Assuming T = 1 day as found by different workers in feeding experiments, than in the interval between 30 and 60 min. the decline in the specific activity should be about 3% only. We assumed in the above calculation that all formation of labeled fatty acid molecules ceases after the lapse of 30 min. As this assumption does not hold strictly, the decline in the specific activity of fatty acids in the intervall between 30 and 60 min. is even less than 3%.

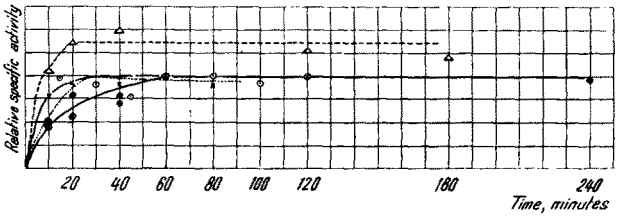

Fig. 2 – Change of the specific activity of the brain fatty acids with time.

The data of figure 1 indicate a very much larger decrease in the specific activity of the fatty acids than 3% in the interval between 30 and 60 min. after administration of the labeled acetate. The decrease amounts to about 50%. These results suggest the presence of a rapidly renewable fatty acid fraction in the liver of the mouse.

In experiments in which the labeled acetate is fed for days and the specific activity of the fatty acids in the liver daily determined, the presence of a minor fraction of rapid renewal rate cannot be expected to be observed.

We investigated also the change of the specific activity of the brain fatty acids and muscle fatty acids with time. The specific activity of the brain fatty acids was found, as seen in figure 2, to increase rapidly with time

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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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The authors gratefully acknowledge the assistance of Miss GRACE DE ELLIOTT and Mrs. VARDE PUTTE.

Pharmaceutical Institute of the University of Gent and Institute for Research in Organic Chemistry, University of Stockholm, October 12, 1950.

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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