Abbildung 2 zeigt die Atmung, Phosphorylierung und den P/O-Quotienten von isolierten Mitochondrien des Ehrlich-Mäuse-Ascites-Tumors unter der Einwirkung geringer Konzentrationen von Chlorpromazin.

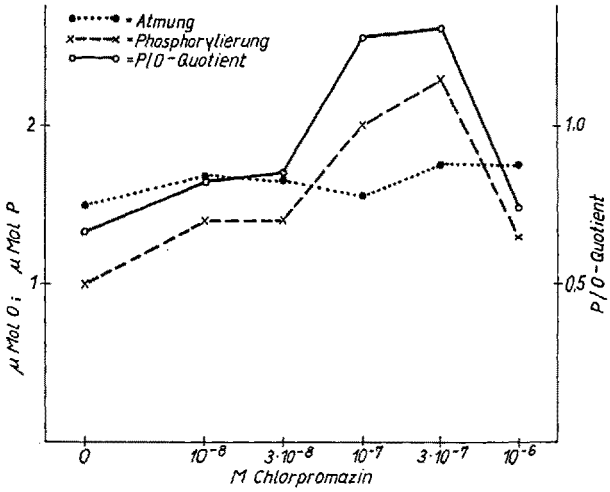

Abb. 2. Einfluss von Chlorpromazin auf die Atmung, Phosphorylierung und den P/O-Quotienten von Mäuse-Ascites-Tumor-Mitochondrien. Medium: 50 µ*M* Phosphat; 60 µ*M* $MgCl_2$; 30 µ*M* NaF; 75 µ*M* Glukose; 0,2 mg Hexokinase; 5 µ*M* ATP; 0,02 µ*M* Zytochrom c; 3 µ*M* DPN; 30 µ*M*-α-Ketoglutarsäure. 0,3 ml Mitochondrien (1,2 mg N) in 0,25 *M* Zucker- und 0,002 *M* EDTA-Lösung; pH 7,0. Endvolumen: 3,0 ml; Temperatur: 30°C; Zeit: 20 min.

Interessant war das Ergebnis einer gleichen Versuchsanordnung bei isolierten Mitochondrien des Walker-Karzinoms der Ratte (Abb. 3). Hier fand sich eine unterschiedliche Wirkung höherer Konzentrationen des Chlorpromazins gegenüber der Stoffwechselwirkung auf normale Lebermitochondrien (siehe Abb. 1 zum Vergleich).

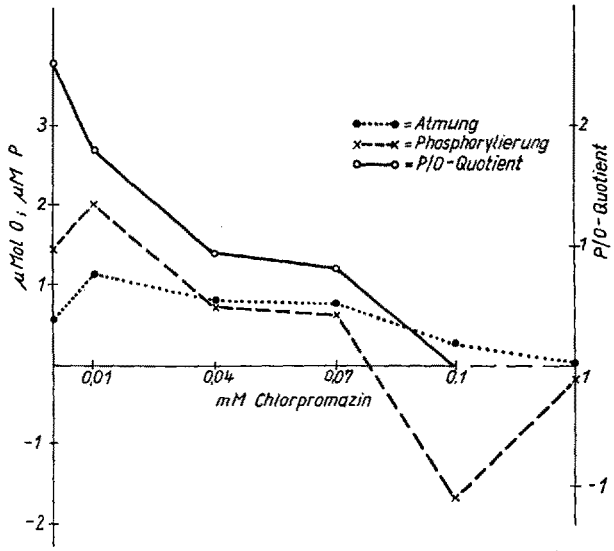

Abb. 3. Einfluss von Chlorpromazin auf die Atmung, Phosphorylierung und den P/O-Quotienten von Walker-Karzinom-Mitochondrien. Bedingungen wie in Abbildung 1.

Die in den Abbildungen verwendeten Abkürzungen sind: ATP = Adenosin Triphosphorsäure; ADP = Adenosin Diphosphorsäure; DPN = Diphosphor-Pyridin-Nukleotid; EDTA = Äthylen-Diamin-Tetraessigsäure.

Man kann aus diesen Ergebnissen folgern, dass durch höhere Konzentrationen von Chlorpromazin der P/O-Quotient der Tumormitochondrien wesentlich stärker herabgesetzt wird als der von normalen Lebermitochondrien. *In vivo* bei transplantierbaren Tiertumoren erhielten wir Ergebnisse, die unsere *in-vitro*-Befunde bestätigen. Hierüber wird an anderer Stelle berichtet. Gleiche Ergebnisse wurden unter gleichen Versuchsbedingungen mit einer anderen entkoppelnden[8] Substanz, dem Atebrin, erhalten.

Auf Grund unserer ersten klinischen Erfahrungen bei der kombinierten Behandlung des humanen Krebses mit Cytostatica oder Bestrahlung möchten wir folgern, dass Tumor-Mitochondrien gegenüber denen von gesunden, auch proliferierenden Mausergeweben eine grössere Beeinflussbarkeit durch Stoffe zeigen, die die oxydative Phosphorylierung je nach Konzentration steigern oder mindern können. Für das Chlorpromazin möchten wir feststellen, dass hohe Konzentrationen dieses Stoffes durch die nachweisbare Entkopplung der oxydativen Phosphorylierung die schon energetisch schwache Tumorzelle in ein Energiedefizit versetzen, so dass zusätzlich verabreichte Cytostatica oder energiereiche Strahlen selektiver wirken können. Beim krebskranken Menschen wirken sich die bekannten anderen pharmakologischen Effekte des Chlorpromazins günstig aus.

W. Lührs, G. Bacigalupo,
B. Kadenbach und E. Heise

Abteilung für experimentelle und klinische Chemo-Therapie der Geschwulstklinik des Institutes für Medizin und Biologie der Deutschen Akademie der Wissenschaften in Berlin-Buch.
12. Mai 1959.

Summary

High concentrations of chlorpromazine cause a considerable decrease of the oxidative phosphorylation of tumor mitochondria and rat liver mitochondria. Regarding preliminary experimental and clinical investigations, we assume that malignant tumors can be sensitised to cytotoxic substances and ionising radiations impairing their energy metabolism by uncoupling the oxidative phosphorylation.

[8] H. Löw, Biochim. biophys. Acta *32*, 1 (1959).

Immune Electrophoretic Analysis of Bovine Milk and Purified Bovine Milk Protein Fractions

The analysis of proteins by means of diffusion-in-gel techniques has been shown to be advantageous because of the sensitivity and specificity of these methods[1]. As a part of a study of bovine milk proteins from chemical and immunological points of view an investigation of the antigenic composition of bovine milk and some purified proteins from milk has been undertaken by means of the Ouchterlony plate technique and immune electrophoresis. Similar studies on human milk have recently been published by Hanson and Johansson[2–4].

Mature bovine milk was used throughout this study. Immune sera against the milk were obtained from hyper-

[1] P. Grabar, Adv. Protein Chem. *13*, 1 (1958).
[2] B. Johansson, Nature *181*, 996 (1958).
[3] L. Å. Hanson and B. Johansson, Int. Arch. Allergy (in press).
[4] L. Å. Hanson, Int. Arch. Allergy (in press).

immunized rabbits. Immune electrophoretic analyses were performed according to GRABAR and WILLIAMS[5]. In some instances the micro-modification described by SCHEIDEGGER[6] was used. Controls of absorption experiments were performed with the micro-slide technique of WADSWORTH[7].

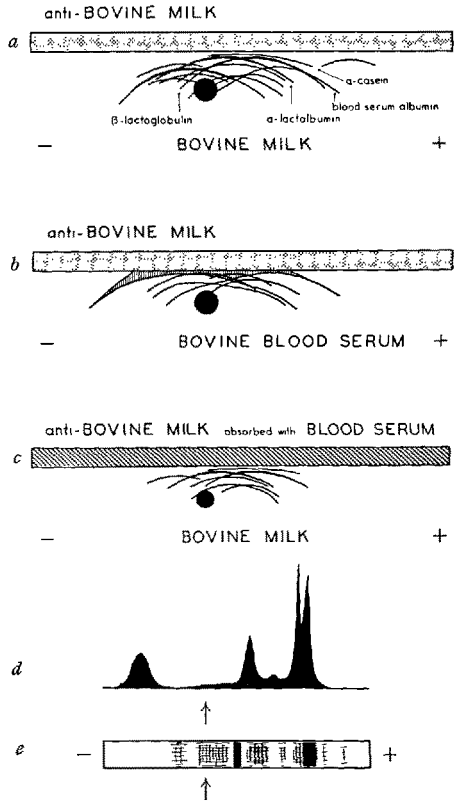

Fig. 1

a) Diagram of immune electrophoretic analysis of bovine milk by means of anti-bovine milk serum.
b) Diagram of immune electrophoretic analysis of bovine blood serum by means of anti-bovine milk serum.
c) Diagram of immune electrophoretic analysis of bovine milk by means of anti-bovine milk serum absorbed with bovine blood serum.
d) Moving boundary electrophoresis of bovine milk.
e) Agar gel electrophoresis of bovine milk.

The milk-anti-milk spectrum obtained in an immune electrophoretic experiment is shown in Figure 1*a*. With the immune serum used, twelve separate precipitates were formed. The relative mobilities of the antigenic factors varied from that of the immune globulins to a mobility higher than serum albumin as determined by the position of the immune precipitates when compared with moving boundary electrophoresis and agar gel electrophoresis of the milk (Fig. 1*d* and *e*). The immune electrophoretic analysis of bovine blood serum by means of the anti-milk serum showed six separate precipitation lines (Fig. 1*b*). These were situated throughout the regions of γ-globulin to serum albumin as shown by comparison with agar gel electrophoresis of bovine blood serum. The presence of antigenic factors in the milk corresponding serologically to blood serum albumin and blood serum γ-globulin was indicated by absorption experiments. Anti-milk immune serum was absorbed with a preparation of blood serum albumin and used to analyze electrophoretically separated milk and blood serum. In both experiments the precipitates observed in the albumin regions in the total spectra (Fig. 1*a* and *b*) had vanished. In a similar manner anti-milk immune serum was absorbed with a preparation of blood serum γ-globulin and tested with electrophoretically separated milk and blood serum. Thus the line found in the γ-globulin region of the total anti-milk-blood serum spectrum (Fig. 1*b*) and the most dense precipitate situated in the immune globulin region of the milk-anti-milk spectrum (Fig. 1*a*) were both absent when this absorbed antiserum was employed.

The anti-milk serum, absorbed with bovine blood serum, showed seven separate precipitation lines in the immune

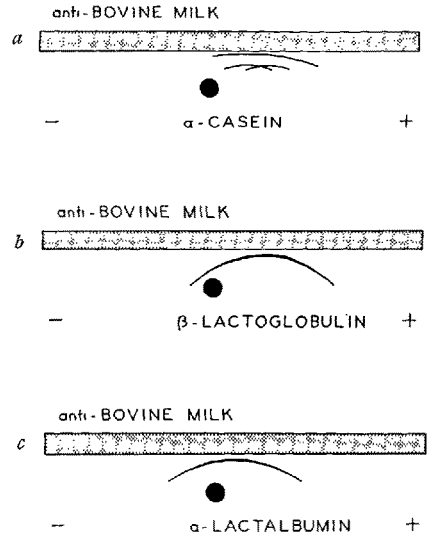

Fig. 2

a) Diagram of immune electrophoretic analysis of α-casein by means of anti-bovine milk serum.
b) Diagram of immune electrophoretic analysis of β-lactoglobulin by means of anti-bovine milk serum.
c) Diagram of immune electrophoretic analysis of α-lactalbumin by means of anti-bovine milk serum.

electrophoretic analysis of bovine milk (Fig. 1*c*). This spectrum was assumed to contain the dominating milk proteins, i.a. α-casein, β-lactoglobulin and α-lactalbumin. In order to identify these proteins in the precipitation spectra, preparations of α-casein[8], crystalline β-lactoglobulin[9], and crystalline α-lactalbumin[9] were made and tested by immune electrophoresis. The patterns formed by these preparations are shown in Figure 2*a–c*. The α-casein preparation gave a long line extending from the starting basin to the albumin region. The line had no distinct maximum. When a higher concentration of α-casein was used two more lines were formed as seen in Figure 2*a*. The β-lactoglobulin and α-lactalbumin preparations each gave one line situated in the regions corresponding to the localization of these proteins in agar gel electrophoresis. Experiments with the double diffusion technique, also showed three lines with α-casein and one line each with β-lactoglobulin and α-lactalbumin. Comparative analysis of the α-casein with blood serum albu-

[5] P. GRABAR and C. A. WILLIAMS, Biochim. biophys. Acta *17*, 67 (1955).
[6] J. J. SCHEIDEGGER, Int. Arch. Allergy *7*, 103 (1955).
[7] C. WADSWORTH, Int. Arch. Allergy *10*, 355 (1957).
[8] N. J. HIPP, M. L. GROVERS, J. H. CUSTER, and T. L. MCMEEKIN, J. Dairy Sci. *35*, 272 (1952).
[9] R. ASCHAFFENBURG and J. DREWRY, Biochem. J. *65*, 273 (1957).

min and β-lactoglobulin by means of anti-milk serum indicated that these three substances were serologically unrelated. However, analysis of α-lactalbumin and β-lactoglobulin with their homologous sera showed more than one precipitate with each protein. By means of absorption experiments the position of the β-lactoglobulin and α-lactalbumin precipitation lines in the total milk-anti-milk spectrum could be established as designated in Figure 1 *a*. In the same way the extended line formed by α-casein could be identified in the total spectrum.

These and further investigations of the immunological characteristics of bovine milk proteins in native and modified forms will be published in detail elsewhere.

L. Å. HANSON and B. JOHANSSON

Departments of Bacteriology and Medical Biochemistry, University of Gothenburg (Sweden), May 6, 1959.

Zusammenfassung

Immunoelektrophoretisch liessen sich aus Kuhmilch mit Hilfe einiger Kaninchen-Anti-Milchseren mindestens 12 Immunpräzipitate nachweisen. Von diesen waren 6 Präzipitate serologisch mit Blutserumproteinen verwandt (u. a. Albumin und γ-Globulin). Ferner wurden Präparate von α-Kasein, β-Laktoglobulin und α-Laktalbumin analysiert.

Catalase Activity in the Regenerating Tail Tip of Xenopus Larvae and the Effect of 3-Amino-1,2,4-triazole[1]

In previous papers[2,3] we were able to show that tail tissue of Xenopus larvae contains a catalase with much the same kinetic properties as catalase from mammalian organs[2]. It is distributed along the tail axis in a steep gradient, the specific activity being highest in the tip and lowest in the base of the tail[3]. Catalase is well known for its marked depression in the livers and kidneys of tumor-bearing rats, whereas it shows normal levels of activity in regenerating rat liver[4]. On the other hand we have found recently that the activity of the intracellular proteases (cathepsins) is strongly increased above the normal level in the regenerating tail tip of Xenopus larvae; it is further increased in regenerates partly inhibited by a morphostatic aminoketone and a quinoxaline derivative[5]. In view of this, it was of interest to follow the behaviour of catalase activity during the process of regeneration in Xenopus larvae. This would extend our knowledge of the enzyme pattern in the regenerating tail tissue and serve as a basis for further tests of the biochemical effects produced by morphostatic substances on our model tissue.

[1] This work was supported by a grant of the *Eidgenössische Kommission zur Förderung der wissenschaftlichen Forschung aus Arbeitsbeschaffungsmitteln des Bundes*. I would like to express my gratitude to Prof. F. E. LEHMANN for his constant interest and his valuable advice. I am also indebted to Mrs. J. WEBER and to Miss M. PIEREN for technical assistance.

[2] H. P. VON HAHN, Helv. chim. Acta *42*, 49 (1959).

[3] H. P. VON HAHN, Exper. *14*, 67 (1958).

[4] J. P. GREENSTEIN, *Biochemistry of Cancer* (Academic Press Inc., Publ., New York 1954), p. 519.

[5] H. P. VON HAHN and F. E. LEHMANN, Helv. physiol. Acta *16*, 107 (1958).

In the first test we determined the effect of 3-amino-1,2,4-triazole (AT), known as a potent catalase inhibitor *in vivo* in the rat[6], on our system.

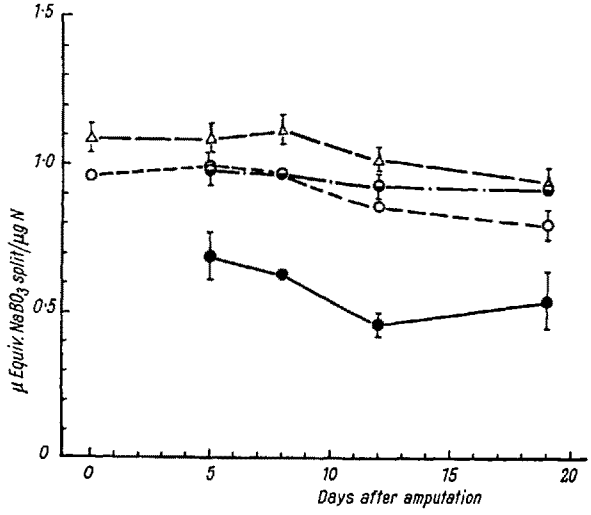

Fig. 1. – Specific catalase activity during the regeneration of the tail tip of Xenopus larvae. • Regenerates, ○ stumpslices, ▽ 0th–7th mm and ○ 7 th–9th mm of the tails of the unamputated controls. The vertical lines indicate the standard error of the determinations.

Methods. We used the micro-adaptation of the perborate test of FEINSTEIN[7] which we recently described[2]. Xenopus larvae, 25 days old and 26–30 mm in overall length, were kept in distilled water at 18°C in a thermostat. In the first group of animals, 7 mm of the tail tip were amputated at the beginning of the experiment (day 0). A second group of non-amputated animals served as a control and was kept under identical conditions. The larvae were not fed throughout the period of the experiment. On the 5th, 8th, 12th, and 19th days after amputation, tissue pieces were collected from at least 10 larvae each, as follows: (a) from the amputated animals, the regenerates and the adjoining 2 mm stump slices; (b) from the non-amputated animals 7 mm slices from the tail tip as controls to the regenerates and 7th–9th mm slices as controls to the stump slices. Catalase activity and total nitrogen (by the ultramicro-Kjeldahl technique of BOELL and SHEN[8]) were determined in homogenates of these 4 tissue pieces.

In the experiment with AT, the amputated larvae were kept in a 1:4000 solution of AT. One group of non-amputated larvae in AT and another in water served as controls. The regenerates and the 2 mm stump sclices were collected from the amputated animals, and the 7th–9th mm slices were collected from the tails of the treated and the untreated controls. The results for both experiments are expressed in microequivalents perborate split per µg total nitrogen (for an incubation time of 5 min).

Results. Specific catalase activity during normal regeneration is shown in Figure 1. In the tails of the non-amputated controls catalase activity remained almost unchanged throughout the experimental period, the tip

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[7] R. N. FEINSTEIN, J. biol. Chem. *180*, 1197 (1949).

[8] E. J. BOELL and S. C. SHEN, Exp. Cell Res. *7*, 147 (1954).