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.

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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.

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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.

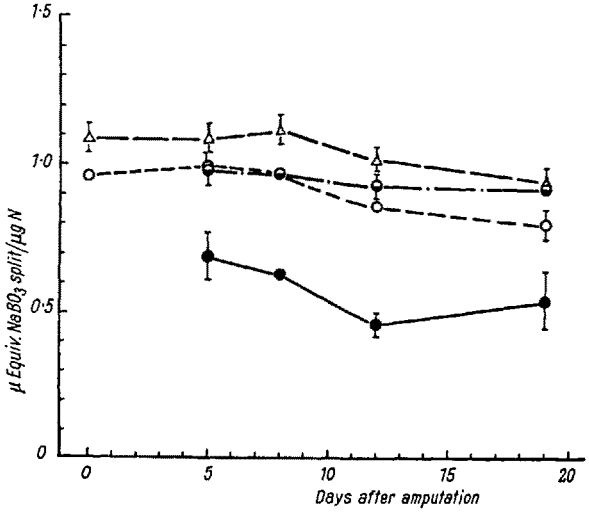

Fig. 1.—Specific catalase activity during the regeneration of the tail tip of Xenopus larvae. • Regenerates, ○ stumpslices, ▽ 0th–7th mm and ○ 7 th–9th mm of the tails of the unamputated controls. The vertical lines indicate the standard error of the determinations.

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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of the tail to the 7th mm showing consistently about 10% more activity than the adjoining 2 mm slice (7th–9th mm). This confirms the gradient-like distribution shown previously[3]. The slight loss of activity in these two tissue pieces from the 8th to the 19th day is not statistically significant. The regenerates, on the other hand, show a marked decrease in catalase activity to about 50% of the level found in the corresponding region of the non-amputated tails (0–7 mm). Only towards the end of the regeneration period does the activity seem to increase again, to a very slight extent. That this decrease is specific for the regenerating tissue is shown by the unchanged level of activity in the adjoining 2 mm stump slices. This behaviour is in contrast to the results found in regenerating rat liver, where catalase activity is not decreased[4]. It is also in contrast to our own results with the cathepsins[5], which in the regenerates return to the normal level within 10 days after amputation, i.e. when the regenerates are fully differentiated and further growth proceeds without gain in nitrogen content[9]. In the case of catalase, the abnormally low level is maintained, and in this respect the regenerates keep their specific characteristics even after differentiation is completed.

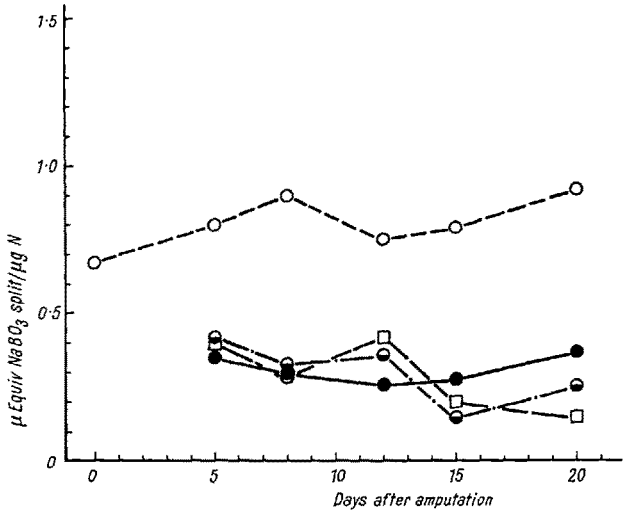

Fig. 2. – Specific catalase activity during regeneration in a 1:4000 solution of AT. • Regenerates, ⊖ stump slices, □ 7th–9th mm of the tails of the unamputated larvae treated with AT, ○ 7th–9th mm of the tails of the untreated controls.

A concentration of 1:4000 of AT inhibited catalase in the tail tissue of amputated and non-amputated larvae (Fig. 2). Both in the regenerates and in the stump slices of the amputated larvae as well as in the 7th–9th mm slices of the treated, non-amputated controls the activity was depressed to about the same level, namely 20–40% of the untreated controls. In the regenerates, catalase activity was decreased to a much smaller extent than in the stump and control slices. Here the level falls only to about 60 to 70% of the untreated regenerates (see Fig. 1). It seems as if at the AT concentration used, a definite minimum level of catalase activity is maintained in the treated tissues.

The regeneration of tail tissue was partly inhibited by AT during the first days after amputation. On the 5th day, AT-treated regenerates measured only 0,55 mm (average of 50) while untreated regenerates measured 1,00 mm (average of 10). But by the 12th day treated regenerates averaged 3,99 mm to 3,78 mm for the untreated, and at the end of the experiment (20th day) the values were 5,20 and 4,45 mm respectively. Thus it seems that those cells in the young blastema which survived the first effects of the treatment retained their full vitality, since the tissue as a whole did not lose any of its regenerative power.

One is led to the conclusion that the blocking of catalase in the regenerate does not in itself affect any vital metabolic pathway, and that the inhibition observed may possibly be due to a contaminating substance with a specific effect on the early phases of regeneration. The presence of such biologically active 'aminotriazole contaminant' has been discussed by FEINSTEIN *et al.*[10]. It should be possible to separate the catalase-blocking and the regeneration-inhibiting components of AT-preparations and thus identify the active agents. Such work is at present in progress and will be reported on later.

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Zusammenfassung

In der regenerierenden Schwanzspitze der Xenopuslarve bleibt die Katalase-Aktivität (bezogen auf Totalstickstoff) bis zum Ende der Regenerationsperiode auf etwa 50% des normalen Niveaus, während sie im anschliessenden Schwanzstumpf unverändert ist. 3-Amino-1,2,4-triazol erniedrigt die Katalase-Aktivität in den Regeneraten, den Stümpfen und dem Kontrollgewebe nicht-amputierter Larven auf etwa 20–40% des normalen Niveaus. Es bewirkt eine kurzfristige Regenerationshemmung in den ersten Tagen nach Amputation, die im weiteren Verlauf wieder vollständig ausgeglichen wird.

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Die Komplement-hemmende Wirkung einiger Antiproteasen

Zum Wirkungsmechanismus des Komplements bei immunologischer Hämolyse gehört eine primäre Aktivierung von C'_1 durch den Antigen-Antikörper-Komplex (eventuell auch eine unspezifische Aktivierung durch die proteolytischen Enzyme Plasmin und Trypsin) zu «aktivem C'_1», welches die Eigenschaften einer Protease mit Esterase-Aktivität aufweisen soll[1–3]. Die Hemmung dieser ersten Stufe und damit des ganzen Komplements könnte für Allergie und Immunohämatologie von grosser Bedeutung sein[3,4]. CUSHMAN *et al.*[5,6] beobachteten die hemmende Wirkung von Substanzen wie *p*-Toluolsulfonyl-l-arginin-methylester, einigen Polypeptiden, Aminosäuren und Diisopropylfluorophosphat; diese waren jedoch entweder zu wenig aktiv oder wegen ihrer Toxizität therapeutisch nicht verwendbar.

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