dem Chromatogramm herausgeschnitten, in ein oben plangeschliffenes Gläschen (ø 2 cm, Höhe 4 cm) übertragen, das Amin mit 0,5 ml 3*n* NaOH freigesetzt und das Gläschen sofort mit einem Deckglas, das in einem Hängetropfen Wasser einige Kristalle DNN-Reagenz enthielt, verschlossen. Nach ca. 24 h waren schön ausgebildete Kristalle der DNN-Aminverbindung entstanden. Auf Grund der charakteristischen Kristallformen, Schmelzbereiche und Umlagerungsprodukte sowie vorliegenden Angaben aus der Literatur[5,6], konnte das Amin sicher als *n*-Butylamin identifiziert werden (Tabelle).

n-Butylamin liess sich bei den Apfelsorten «Cox's Orange», «Golden Delicious», «Winter Glockenapfel», «Jonathan» und «Boskop» nachweisen. Isobutylamin wurde in den angegebenen Apfelsorten nicht gefunden.

Im Gegensatz zu dem sonst im Pflanzenreich verbreitet vorkommenden Isobutylamin[7], ist *n*-Butylamin unseres Wissens in der Natur bisher nicht gefunden worden. Sein Vorkommen in Äpfeln ist im Zusammenhang mit Problemen der Aminbiogenese interessant. Im Gegensatz zu der häufig vertretenen Vorstellung, dass Amine nur durch Decarboxylierung aus den entsprechenden Aminosäuren gebildet werden, konnte für Äpfel eine Aminbildung durch Aldehydaminierung wahrscheinlich gemacht werden[2]. *n*-Butyraldehyd, die zu vermutende Vorstufe des *n*-Butylamins, wurde in Apfelexhalaten nachgewiesen[8,9].

Summary. *n*-Butylamine, a new biogenic amine could be identified in apples by means of paper chromatography and micro-cristallography.

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Additional Studies on the Effects of Neonatal Thymectomy and Lactate Dehydrogenase Virus Infection on Mice[1]

In a previous report[2] it was shown that serum lactate dehydrogenase (LDH) levels and LDH virus titers were higher in 5-week-old mice thymectomized at birth and infected thereafter with the LDH virus than in infected, but non-operated, mice of the same age. The studies now described were undertaken to determine the effect(s) of neonatal thymectomy on: (a) the growth rate and lifespan of infected animals; (b) the immunologic response in mice to the LDH virus; and (c) the pathogenicity of the LDH virus.

Materials and methods. Adult, female LCS/Fg mice received an i.p. injection (0.1 ml/mouse) of $10^{7.5}ID_{50}$/ml of virus before conception or during gestation[3]. Within 12–24 h after birth, offspring from these 2 groups of animals were sham-operated or thymectomized by the method of SJODIN et al.[4]. In a third group, babies born to uninfected mothers were: (a) neonatally thymectomized and infected[5]; (b) neonatally thymectomized, but not infected[6]; (c) sham-operated and infected[5]; or (d) sham-operated, but not infected[6]. Pregnant females, as well as mothers and babies, were treated as described previously[2].

All progeny were weighed at 1 week of age and thereafter, at 6-day intervals, until 13 weeks old. During this period, at 5 weeks after birth, all offspring were weaned and isolated, according to sex and treatment, in separate cages. Concurrently, blood was collected from each animal by tail bleeding; plasma LDH levels were determined as described elsewhere[3]. In addition, aliquots (0.1 ml) of these plasma samples, obtained by pooling specimens from 3–4 animals in the same experimental or control group, were incubated at 50 °C for 60 min with an equal volume of phosphate-buffered saline (PBS), pH 7.2, or 2*M* $MgCl_2$[7]. Thereafter, serial tenfold dilutions of these test materials were injected i.p. (0.1 ml/mouse) into recipient mice. 1 week later, the test animals were bled, their plasma assayed for LDH activity, and infective titers calculated by the method of REED and MUENCH[8].

Animal cages were inspected daily for dead mice which were removed and examined carefully, both for residual thymic tissue and gross pathologic lesions. Mice still alive at 20 weeks of age were killed; similar observations were made and recorded. At this time, data collected from neonatally thymectomized mice were further subdivided in terms of whether or not pieces of thymus were seen at necropsy (partial or complete thymectomy).

Results. The observation that plasma LDH levels in thymectomized (complete), infected mice were 2–4 times higher than the enzyme activities observed in the plasma of sham-operated, infected animals at 5 weeks of age (Table I) provides confirmation of results which we reported in an earlier paper[2]. Since data obtained from sham-operated, infected and thymectomized (partial), infected mice were similar (Table I), it would appear that plasma LDH levels are not affected significantly if neonatal thymectomy is not complete. It should also be noted that infection of females before conception or during gestation and of babies after birth had no apparent

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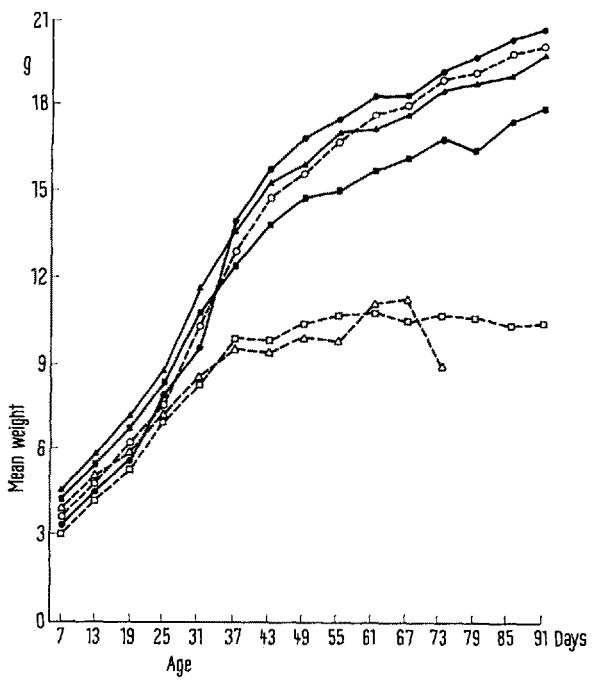
effect on the plasma LDH levels within each of the 3 groups of offspring (sham-operated, partial thymectomy, or complete thymectomy) tested.

Although gross pathologic lesions were not observed in either experimental or control animals, the possibility of an altered response in mice to neonatal thymectomy as a consequence of LDH virus infection is suggested by the data summarized in Table II. Specifically, whereas all of the thymectomized (complete), non-infected offspring were dead by 11 weeks of age, 12% of the thymectomized (complete), infected progeny were still alive at 20 weeks after birth. A similar relationship was also found between the 2 groups of partially thymectomized animals in that 97% of the infected mice, as compared to 77% of the non-infected babies, survived until 20 weeks of age. Additional studies are now in progress to determine the possible significance of these findings. In this regard, it is of interest to note the recent report of a decrease in the incidence of spontaneous mammary tumors among mice infected with the LDH virus[9].

The weight curves of mice in the 6 experimental and control groups used in this study are shown in the Figure. It can be seen that the mean weights of thymectomized (complete) animals, whether infected or non-infected, were decreased significantly and did not exceed 11.3 g



Effect of neonatal thymectomy and/or infection with the lactate dehydrogenase virus on the weight increase of mice. ●——● sham-operated, non-infected; o----o sham-operated, infected; ▲——▲ thymectomized (partial), non-infected; ■——■ thymectomized (partial), infected; △----△ thymectomized (complete), non-infected; □----□ thymectomized (complete), infected.

Table I. Effect of neonatal thymectomy and/or infection with the lactate dehydrogenase (LDH) virus on plasma LDH levels in 5-week-old mice

Treatment	No. of mice tested	Plasma LDH (units/ml)	
		Range	Mean
Sham-operated; non-infected	18	300– 1300	850
Thymectomized (partial); non-infected	17	400– 1300	900
Thymectomized (complete); non-infected	11	400– 1200	900
Sham-operated; infected[a]	41	2800– 5100	3950
Thymectomized (partial); infected[a]	31	3200– 6000	4850
Thymectomized (complete); infected[a]	37	6600–11,000	8100

[a] Data obtained from babies infected within 48 h after birth and the offspring of females infected before conception or during gestation were similar and have been combined.

Table II. Effect of neonatal thymectomy and/or infection with the lactate dehydrogenase (LDH) virus on the survival of mice until 20 weeks of age

Treatment	No. of mice tested	Alive at 20 weeks	
		No.	%
Sham-operated; non-infected	20	18	90
Thymectomized (partial); non-infected	17	13	77
Thymectomized (complete); non-infected	11	0	0
Sham-operated; infected[a]	50	47	94
Thymectomized (partial); infected[a]	31	30	97
Thymectomized (complete); infected[a]	42	5	12

[a] Data obtained from babies infected within 48 h after birth and the offspring of females infected before conception or during gestation were similar and have been combined.

Table III. Effect of neonatal thymectomy and/or time of infection with the lactate dehydrogenase (LDH) virus on virus titers in 5-week-old mice

Treatment		ID_{50}/ml (log 10)[a]	
Mother	Babies	PBS[b]	$MgCl_2$[c]
Infected before conception	Sham-operated	7.4	5.4
Infected before conception	Thymectomized (complete)	7.4	5.5
Infected during gestation	Sham-operated	7.3	5.4
Infected during gestation	Thymectomized (complete)	7.5	5.5
Non-infected	Sham-operated; infected	7.3	5.4
Non-infected	Thymectomized (complete); infected	8.3	5.4

[a] Each value represents the mean of 3 separate titrations. [b] Plasma incubated at 50 °C for 60 min with an equal volume of phosphate-buffered saline, pH 7.2. [c] Plasma incubated at 50 °C for 60 min with an equal volume of 2 *M* $MgCl_2$.

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during the test period. Of particular interest was the finding that the increase in mean weights of sham-operated, infected and thymectomized (partial), non-infected animals was less than that observed in sham-operated, non-infected mice. Moreover, there was a still greater reduction in the mean weights of thymectomized (partial), infected animals. Thus, it would appear that partial thymectomy or infection with the LDH virus have an effect on mouse weight and when both factors are present in the same animal these effects are additive.

The results shown in Table III indicate that: (a) plasma virus titers ($MgCl_2$-resistant fraction) were not affected by neonatal thymectomy or time of infection; (b) the total virus titer (PBS) in the plasma of 5-week-old mice, thymectomized and infected after birth, was higher than the mean infective titer observed in sham-operated, infected animals of the same age; and (c) plasma virus titers (PBS) in sham-operated or neonatally thymectomized offspring born to mothers infected before conception or during gestation were similar. These findings are presented as additional evidence in support of a model system involving 2 particles: (a) a smaller virus or S particle which is resistant to heat inactivation in the presence of $MgCl_2$ and non-antigenic; and (b) a larger virus or L particle which is susceptible to heat inactivation in the presence of magnesium ions and antigenic[2,7,10].

Zusammenfassung. Die gegebenen Befunde lassen vermuten, dass neonatale Thymektomie und/oder Infektion mit LDH-Virus einen Effekt auf eine Anzahl von Faktoren wie LDH-Plasma-Spiegel, Gewichtserhöhung und Lebensdauer in Mäusen hervorrufen können. Der Virustiter im Plasma ist vom Zeitpunkt der Infektion abhängig.

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STUDIORUM PROGRESSUS

Heterogeneity of Lactate Dehydrogenase in the Developing and Adult *Xenopus laevis* Daud[1]

Experiments to determine the isoenzymes of amphibian lactate dehydrogenase (LDH) showed either 2 distinct components[2,3], or 4[4], as opposed to the pattern of 5 isoenzymes found in most vertebrate classes. These 5 components have shown to be tetramers resulting from the combination of 2 types of protein subunits designated as A and B, or H and M, and the synthesis of the 2 different polypeptide subunits is regarded as being under the control of separate genes[5].

Xenopus laevis was selected because of its unique larval development, which shows at least 20 differences, most of them radical, from the typical anuran pattern[6]. An extensive study of the LDH development, from fertilized egg to sexually mature adult, has been in progress in this department. Results proved so challenging that it was decided to give this preliminary report.

The adult (6 years old) animals were taken from laboratory stock. To examine the developmental stages, toads were injected with 400 IU of the choriogonadotropic hormone Pregnyl (Organon). The spawns, thus obtained, were raised at 22 °C; the food used was nettle powder (Herba urticae) during the larval period and ox liver after metamorphosis. Unfertilized eggs were produced by injecting isolated females with the same hormone. The LDH-isoenzymes were separated electrophoretically on agar-gel using the high voltage technique of WIEME[7] (pp. 68, 148, 157) and on starch-gel, as previously described[3], and subsequently stained with iodoneotetrazolium (INT) and Neotetrazolium respectively. Samples were applied in various ways. Agar-gel: (a) Specimens were homogenized in twice their volume of bi-distilled water, centrifuged under cooling for 20 min at 16,000 rpm and 3 λ of the supernatant applied to a narrow slit. (b) Small fragments were pushed into a cut made in the gel (direct tissue electrophoresis)[7]. Starch-gel: Samples were homogenized in *Tris* buffer, pH 8.9, centrifuged for 20 min at 16,000 rpm at 7 °C, and the supernatant placed on a filter paper strip and inserted into the gel.

In the results (Figures 1 and 2) the relative activity of the isoenzyme bands is expressed by shading, and the bands are numbered from 1–9 in their order of migration towards the anode. -Agar-gel: 9 bands were observed in organs taken from the adult toad (the only exception being the liver), the corresponding bands in each occupying the same position but differing in strength (Figure 2). Changes observed on total homogenates during development are the following. Unfertilized eggs, extruded into water, yield bands 1 and 2. Fertilized eggs at the 2-cell stage, up to neurulation (day 1 and 2), show a pattern of 4 bands – No. 1–4. At the tailbud stage (end of 2nd day) band 9 makes its appearance. During the following 2 days (day 3–4) – which are characterized by the outgrowth of the tail, pigmentation of eyes, appearance of external gills, beginning of heart beat and hatching of the embryo – band 8 shows up; bands 2, 3 and 4 disappear temporarily but 2 and 4 reappear within 1 day. From the 5th–7th day, the external gills are absorbed and replaced by internal gills; the animals previously attached by cement glands

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