males were fed for 3 days with the test solutions and were then mated to the same number of untreated virgin females of the same age kept in cages with untreated milk and sugar. Eggs were collected daily and measured volumetrically, and hatching of eggs was checked on at least 6 occasions up to the age of 14 days of the females; the experiments were conducted at 27 °C. Some representative results, which could be confirmed in several replications, are given in the Table. The results indicate that while *m*-XHQ dissolved in water was inactive as a chemosterilant, *m*-XHQ with vitamin C in aqueous solution was as active as when dissolved in milk. Since, as mentioned above, the compound deteriorates also on dry storage, some further experiments were undertaken with strongly oxidized samples of already lilac colour (m.p. 127–128° instead of 150 °C of the pure substance, which is white). Such decomposed samples were nearly inactive when tested dissolved in milk at 0.15%, but when dissolved at the same concentration in water containing 0.3% vitamin C with formation of a colourless solution, they were reduced and reasonably if not completely reactivated (average corrected egg fertility, 12.5%).

We are unable to say whether this stabilization[15] would improve the antifertility effect of *m*-XHQ in mammals, which appears to be 'undramatic and somewhat difficult to demonstrate'[16], but perhaps endocrinologists might find it worthwhile to retest *m*-XHQ mixed with several times its weight of vitamin C, in rats[17,18].

Zusammenfassung. Nachweis, dass sich *m*-Xylohydrochinon (*m*-XHQ; 2,6-Dimethylhydrochinon) in wässriger Lösung zersetzt und so seine Wirkung als Insekten-Chemosterilant verliert. Auflösung der Substanz in Vitamin C enthaltenden wässrigen Lösungen verhindert den Vorgang.

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[15] According to S. N. SANYAL and S. S. GUHA SIRCAR, Med. Int. *27*, 53 (1963) the oral contraceptive *m*-XHQ tablets dispensed by the Indian Government Health Centres contain 'some citric acid used as antioxidant preservative'. We found that when *m*-XHQ was dissolved at a concentration of 0.15% in aqueous solutions of 0.075%, 0.15%, 0.2% or 0.3% citric acid, the solutions turned yellow nearly immediately and developed a strong smell. Within 1 day the yellow colour had become very intense. It was concluded that citric acid is unable to prevent the oxidation of *m*-XHQ in aqueous solutions. Little protection was afforded to *m*-XHQ in water by various mono- and disaccharides. Even a saturated (13:7) sucrose solution in water could not prevent the rapid oxidation of *m*-XHQ dissolved in it, but the protective effect of vitamin C was prolonged when a saturated sucrose solution was used as the vehicle.

[16] H. JACKSON, Pharmac. Rev. *11*, 135 (1959).

[17] We wish to thank Dr. S. N. SANYAL, Calcutta, for numerous samples of *m*-xylohydroquinone.

[18] Contribution from The National and University Institute of Agriculture, Rehovot, Israel. 1967 Series, No. 1126-E.

Nachweis von *n*-Butylamin in Äpfeln

Über das Vorkommen von wasserdampfflüchtigen primären Aminen in Apfelfrüchten berichteten HILKENBÄUMER et al.[1]. Bei den Apfelsorten «Cox's Orange», «Ontario» und «Jonathan» konnten Äthyl-, Isoamyl- und Hexylamin nachgewiesen werden.

Bei eigenen Versuchen über die Biogenese von flüchtigen Aminen konnten wir das Vorkommen von Äthyl- und Hexylamin bestätigen[2]. Isoamylamin dagegen liess sich nicht mit Sicherheit identifizieren. Nur in Einzelfällen, bei den Apfelsorten «Cox's Orange» und «Golden Delicious», konnte neben sehr geringen Mengen von vermutlich Propylamin und einem höheren Homologen des Hexylamins chromatographisch eine ninhydrinpositive Substanz in Spuren nachgewiesen werden, deren Rf-Wert mit dem von Isoamylamin übereinstimmte. Eine sichere Identifizierung war wegen der geringen Mengen nicht möglich. Ausserdem fanden wir ein zunächst unbekanntes Amin, das bei der papierchromatographischen Aufarbeitung (Trennung an Na-Acetat – vorbehandeltem Papier; Fliessmittel: *n*-Butanol-Wasser-Eisessig 50:49:1 (A) bzw. 50:40:10 (B))[3] einen geringfügig aber konstant höheren Rf-Wert als Isobutylamin aufwies und in allen Eigenschaften mit *n*-Butylamin übereinstimmte. Zur sicheren Identifizierung wurde das Amin im Vergleich zu den Butylaminen mikrokristallographisch nach der von KLEIN und STEINER entwickelten Methode mit 2,4-Dinitro-α-naphthol (DNN) untersucht[3–6]: Nach chromatographischer Trennung im Fliessmittelsystem A wurde die interessierende Zone, die ca. 10–20 µg Amin enthielt, aus

Chromatographische und mikrokristallographische Daten der Butylamine im Vergleich zu dem Amin aus Äpfeln

Amin	Rf-Werte		DNN-Verbindungen	
	A	B	Schmelzbereich[a]	Kristallform
Isobutylamin	0,60	0,72	155°–159 °C	Rhomben, prismatische Platten, Tafeln
n-Butylamin	0,66	0,76	147°–153 °C	Oft gekrümmte, lange Nadeln
«Apfelamin»	0,65	0,76	147°–152 °C	Wie *n*-Butylamin

[a] Die Schmelzpunkte variieren auch unter gleichen Bedingungen bei mehreren Einzelbestimmungen über einige Grade. Die aufgeführten Schmelzbereiche ergaben sich auf Grund von je 8 Einzelbestimmungen. Verwendete Apparatur zur Schmelzpunktbestimmung: Mikroskopheiztisch 350 (Leitz).

[1] F. HILKENBÄUMER, G. BUCHLOH und A. ZACHARIAE, Angew. Bot. *34*, 105 (1960).

[2] T. HARTMANN, Z. PflPhysiol., im Druck.

[3] E. STEIN v. KAMIENSKI, Diss. Bonn (1955).

[4] E. STEIN v. KAMIENSKI, Planta *50*, 291 (1957).

[5] G. KLEIN und M. STEINER, Jb. wiss. Bot. *68*, 602 (1928).

[6] B. ZORN und W. DIEHLMANN, Dte GesundhWes. *8*, 1522 (1953).

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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Pharmakognostisches Institut der Universität, 53 Bonn (Deutschland), 22. Februar 1967.

[7] E. STEIN v. KAMIENSKI, Planta *50*, 315 (1957).
[8] D. F. MEIGH, J. Sci. Fd Agric. *7*, 396 (1956).
[9] Für das freundlicherweise zur Verfügung gestellte Apfelmaterial danken wir Herrn Professor Dr. F. HILKENBÄUMER, Institut für Obstbau der Universität Bonn, für die Gewährung von Sachbeihilfen der Deutschen Forschungsgemeinschaft.

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

[1] Supported by Public Health Service research grant No. 5 R01 CA05742-06 from the National Cancer Institute.
[2] C. G. CRISPENS JR., J. natn. Cancer Inst. *36*, 81 (1966)
[3] C. G. CRISPENS JR., J. natn. Cancer Inst. *34*, 331 (1965).
[4] K. SJODIN, A. P. DALMASSO, J. M. SMITH and C. MARTINEZ, Transplantation *1*, 521 (1963).
[5] Each baby received a s.c. injection (0.05 ml/mouse) of plasma containing $10^{7.5}ID_{50}$/ml of virus within 48 h after birth.
[6] Each baby received a s.c. injection (0.05 ml/mouse) of phosphate-buffered saline, pH 7.2, within 48 h after birth.
[7] D. M. STARK and C. G. CRISPENS JR., Experientia *20*, 270 (1965).
[8] L. J. REED and H. MUENCH, Am. J. Hyg. *27*, 493 (1938).