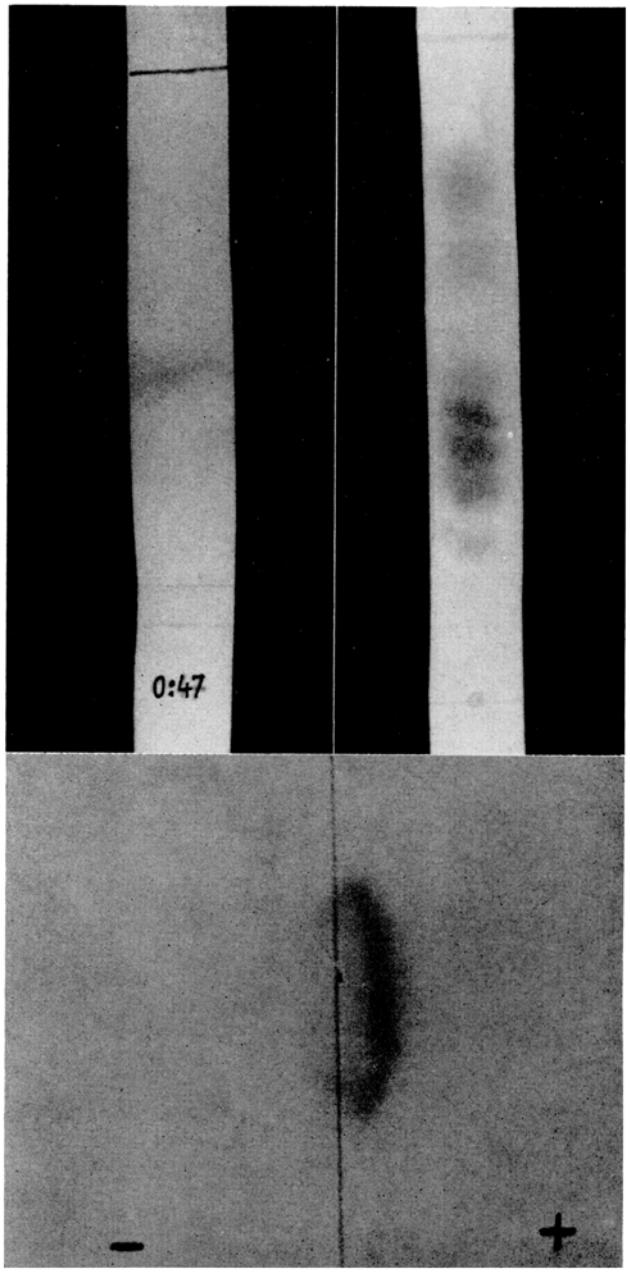

Fig. 2. Links oben: Papierchromatogramm von 0,01 ml Peptid-Lösung. Entwicklungssystem Butanol-Essigsäure-Wasser. Rechts oben: Papierchromatogramm des partiellen Hydrolysats des Peptids; Entwicklungssystem dasselbe. Unten: Elektropherogramm der Peptid-Lösung; 0,02 ml, 300 V, Essigsäurepuffer von pH 1,7.
Bemerkung: Alle Photos prom. Biologe Crha.

Azetonpräzipitatlösung ausgeprägter als nach der Verabreichung von Spermaextraktinjektionen. Übersichtlich sind die makroskopischen und mikroskopischen Veränderungen der Ovarien in der Figur 1 dargestellt. Inaktivation des Spermaextraktes während 1 h durch 90–95°C beeinflusst die biologische Wirksamkeit nur geringfügig.

Obwohl zwischen der Gruppe, die mit FSH behandelt wurde, und der Gruppe, welche mit Spermaextrakt oder Azetonpräzipitatlösung behandelt wurde, histologisch einige Differenzen bemerkbar sind, finden wir in beiden Reihen Stimulation des Follikelwachstums, Beschleunigung des Ovarienwachstums und der Vermehrung der Granulosazellen, also jene Erscheinungen, die für die Wirkung von FSH typisch sind. Ausser diesen biologischen Wirkungen konnten wir auch eine ziemlich starke Nebennierenhypertrophie beobachten und in einigen Fällen eine Verkleinerung der Hypophyse. Ob diese Veränderungen spezifisch sind, müssen jetzt weitere Versuche abklären.

Obwohl der in Spermaextrakten anwesende Stoff biologisch dem FSH nahe steht, ist er mit diesem Hormon nicht identisch. Die papierelektrophoretischen und papierchromatographischen Studien zeigten, dass es sich um ein Peptid handelt. Das Peptid hat ungefähr 13 Aminosäurereste, enthält einen Zucker (positive Molisch-Reaktion); seine biologische Aktivität beträgt ungefähr 35 Einheiten/g. Chromatographisch und elektrophoretisch verhielt sich der Stoff einheitlich (Figur 2). Unseren Schätzungen nach enthalten 100 ml Ebersperma ungefähr 0,04–0,05% dieses Stoffes.

In den Vordergrund treten nun einige Fragen: (1) ob die beschriebene Wirkung direkt oder indirekt ist, (2) welchen Ursprungs dieser Stoff im Sperma ist. Eine Beantwortung dieser Fragen können nur weitere Versuche ergeben. Wir hoffen aber, dass der Befund eines biologisch wirksamen Peptids im Sperma einige Befunde anderer Forscher näher klären kann; in erster Linie die Angaben von STRATMAN und SELF[8], DOKUNIN[9] und PITKJANEN[10], sowie auch die Angaben von LAMOND und CLARINGBOLD[11].

Summary. The action of sperm extracts and acetone precipitate of these extracts upon the growth of ovary and uterus was investigated on infantile mice and rats. It was found that injections of the above-mentioned agents increased the uterine and ovarian weights and caused microscopic changes similar to those caused by FSH. The active principle present in the sperm must be a peptid.

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Nitrosamines and Other Carcinogens as Agents of Protein Denaturation[1,2]

The hypothesis that a relationship exists between carcinogenesis and protein denaturation was set forth as early as 1938 by RONDONI (reviewed in [3]). During denaturation a protein molecule passes from a specific configuration to a less orderly structure, while similarly in carcinogenesis there is an increasing anaplasia in the tissue. AMBROSE[4] has pointed out a more direct connection between dedifferentiation of tissue morphology at the microscopic level and submicroscopic alterations at the macromolecular level. Similar concepts have been reached

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by other authors[5–7]. The present report gives the first direct demonstration that carcinogens are agents of protein denaturation.

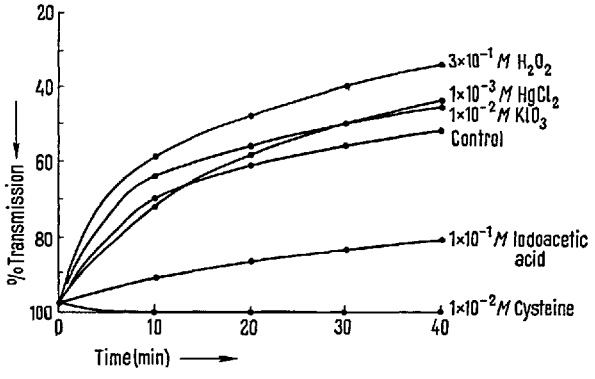
Using turbidity increase of protein solutions to measure denaturation, the results given in the Table indicate that the known water-soluble carcinogens tested here are potent protein denaturants. Diethylnitrosamine, ethyl carbamate and dimethylnitrosamine were also found to precipitate bovine serum albumin in the same relative order of activity as found with ovalbumin. Following the method given in the Table it was found that bovine and human serum albumin, and mercaptalbumin are more resistant than ovalbumin to this type of denaturation produced by dimethylnitrosamine.

This denaturation, measured by turbidimetry, differs from that obtained with urea which causes the disappearance of the native three-dimensional structure of proteins by unfolding the polypeptide chains. Protein denaturation by urea is not accompanied by turbidity increase, but coincides with an increase of optical rotation of the solution. For turbidity increase to take place molecular aggregation must occur involving intermolecular bonds. The Figure summarizes a series of experiments which strongly suggest that the formation of disulfide bridges, by -SH groups made available as a result of unfolding of the protein structure by agents listed in the Table, is a type of intermolecular bond involved here[12]. Mercuric chloride which is known to form –S–Hg–S– bridges, and the oxidizing agents, hydrogen peroxide and potassium iodate, which would favor the formation of disulfide bridges, were all found to enhance the precipitation of ovalbumin by dimethylnitrosamine. None of these agents alone, however, initiate the precipitation of ovalbumin. Iodoacetic acid inhibits the precipitation of ovalbumin by reacting with –SH groups and thus blocking the formation of disulfide bridges. Cysteine, which splits disulfide bonds by virtue of its reducing ability, completely inhibits the precipitation of ovalbumin by dimethylnitrosamine. Moreover, it was found that when 1×10^{-2} *M* cysteine is added at 40 min it completely reverses the precipitation of ovalbumin by dimethylnitrosamine, tannic acid or phenol. Similar observations were made with glutathione. This agent, although as active as cysteine to inhibit precipitation, is less active in reversing this phenomenon. This may be related to lesser permeability of the molecular aggregates to glutathione than to cysteine because of the greater molecular size of the former.

Although urea does not cause precipitation of ovalbumin (Table), it is capable of both enhancing and inhibiting the precipitation caused by 1.5 *M* dimethylnitrosamine. Concentrations below 3.3 *M* urea cause enhancement, while concentrations above 5 *M* cause inhibition. Since the extent of urea-induced unfolding of the polypeptide chains in proteins depends on concentration, the ambivalent effect of urea noted in the turbidity studies may be explained by the fact that disulfide bridges are probably not the only intermolecular bonds maintaining the molecular aggregates. It is possible that intermolecular hydrogen bonding between other groups around the molecular site of the –SH groups may be involved, reinforcing the disulfide linkages formed. The fact that urea exerts its effect on proteins by breaking hydrogen bonds suggests that the carcinogens tested (Table) interact by means of these same valence forces. Preliminary studies have shown that dimethylnitrosamine and dioxane are indeed capable of forming strong hydrogen bonds. Water solutions of these agents display a considerably greater viscosity (1×10^{-2} $\nu_{max} = 1.36$ for dimethylnitrosamine plus water and 1.78 for dioxane plus water) than dimethyl-

The relative ability of these compounds to cause protein denaturation was measured photometrically at 520 mμ by comparing the molar concentrations necessary to produce a 50% decrease in the % transmission at 40 min of a 1% ovalbumin solution at pH 5. Each value in the Table represents the average of four experiments. The carcinogenic activities ('+' active; '—' inactive) of most compounds were taken from HARTWELL's tables[8]. The carcinogenicity of dimethylnitrosamine shown by MAGEE and BARNES[9], and the relatively greater activity of diethylnitrosamine reported by SCHMÄHL and PREUSSMANN[10] have been confirmed in our laboratory[2]. Dimethylhydrazine proved to be inactive as a carcinogen[2]. HOHL has shown that at identical concentrations the carcinogen, ethyl carbamate, and dioxane produce similar chromosomal abnormalities in plant roots[11].

Compound	Molarity necessary to bring about 50% decrease in % transmission of 1% ovalbumin	Carcinogenicity
Tannic acid	0.001	+
Phenol	0.077	+
Diethylnitrosamine	0.440	+
Thioacetamide	0.730	+
Ethylcarbamate	0.830	+
Diethylacetamide	0.850	Untested
Diethylformamide	0.920	Untested
Thiourea	0.930	+
Dimethylnitrosamine	1.550	+
Dioxane	1.800	?; produces chromosomal abnormalities
Dimethylacetamide	2.330	Untested
Acetone	2.430	– to the skin
Dimethylformamide	2.800	Untested
Ethanol	3.330	+ ?
Acetamide	4.800	Untested
Urea	No precipitation up to 5 *M*	–
Dimethylhydrazine	No precipitation up to 6 *M*	–
Dimethylamine	No precipitation up to 0.25 *M*	Untested
Diethylamine	No precipitation up to 6 *M*	Untested



Effect of sulfhydryl reagents on the precipitation by 1.5 *M* dimethylnitrosamine of a 1% ovalbumin solution, as measured by turbidimetry at 520 mμ.

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nitrosamine, dioxane or water alone (1×10^{-2} $\nu = 0.82$, 1.13, and 0.86 respectively). On the other hand, no such increase is observed in mixtures of dimethylnitrosamine and benzene, or dimethylnitrosamine and dioxane because of the absence of a hydrogen donor.

Dimethylhydrazine (1×10^{-2} $\nu = 0.69$), which is a reduced derivative of dimethylnitrosamine, also forms hydrogen bonds as indicated by a considerable increase of viscosity of water solutions (1×10^{-2} $\nu_{max} = 5.90$), but does not bring about precipitation of ovalbumin (Table). This compound, however, is a strong reducing agent, and thus it was not unexpected when it was found that 0.1 *M* dimethylhydrazine both inhibits and reverses the precipitation of ovalbumin by 1.5 *M* dimethylnitrosamine.

A full account of these and related investigations will be given elsewhere.

Résumé. Les auteurs montrent que les composés cancérogènes hydrosolubles, l'acide tannique, le phénol, la diéthyl- et diméthylnitrosamine, la thioacétamide, le carbamate d'éthyl et la thiourée sont des agents puissants de dénaturation de protéines. La précipitation produite par ces agents peut être inhibée, inversée ou accrue par divers réactifs de groupes sulfhydryles. Ces recoupements, ainsi que d'autres expériences sur l'effet de l'urée sur la précipitation par la diméthylnitrosamine, indiquent que le mécanisme de la formation des aggrégats moléculaires consiste dans l'établissement de ponts –S–S– intermoléculaires, probablement renforcés par des liaisons intermoléculaires d'hydrogène.

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Pathogenesis of the Uremic Syndrome[1]. Pharmacological Studies on Acetoin and 2,3-Butylene Glycol[2]

Most of the uremic manifestations are as yet unexplained. Numerous attempts to relate the symptoms of the uremic syndrome to accumulation of a single toxic substance have failed to receive clinical or experimental substantiation. The mechanisms of Kussmaul's respiration, of hyperkaliemic cardiac arrest and of hypokaliemic intestinal and muscular paresis show that the pathogenesis of the uremic syndrome is very complicated. The uremic disturbances of consciousness are of particular interest. They cannot be differentiated from the hepatic coma. In most cases with uremic disturbances of consciousness, residual-nitrogen in serum is increased. Electrolyte imbalance, water intoxication, ammonia, urea and phenol compounds are not correlated to the disturbances of consciousness in uremia[3–5].

In the blood of patients with uremia or hepatic coma, pyruvic acid is raised[6,7]. In mammals pyruvic acid is decarboxylated to active acetaldehyde[8,9]. Active acetaldehyde is mainly converted to acetyl-coenzyme A or may react with acetaldehyde to give acetoin[8,9]. Acetoin may be reduced to 2,3-butylene glycol. The increase in blood pyruvic acid concentration above the normal range in patients with uremia and hepatic coma is probably due to reduced formation of acetyl-coenzyme A. It might be expected that, as a compensatory mechanism, formation of acetoin and 2,3-butylene glycol is enhanced (Figure 1). Glycols produce narcosis, inflammation, hemolysis and impaired permeability. This paper presents observations on the pharmacology of acetoin and 2,3-butylene glycol.

Methods. Narcotic effects on mice after intraperitoneal injection of acetoin or 2,3-butylene glycol. Potentiation of narcosis by acetoin or 2,3-butylene glycol on mice. Blood pressure: canulation of arteria carotis of white rats; kymographic registration; intravenous injection of acetoin or 2,3-butylene glycol. Rate of respiration of anesthetized white rats; intravenous injection of acetoin or 2,3-butylene glycol. Effects of 2,3-butylene glycol on capillary vessels of the frog's web.

Results. In mice acetoin and 2,3-butylene glycol produce a narcotic effect. Before narcosis, muscular twitching may be observed. Acetoin is about four times more active in producing narcosis than 2,3-butylene glycol. After rapid intravenous injection of acetoin or 2,3-butylene glycol in rats, blood pressure (Figure 2), pulse rate (Figure 3) and rate of respiration decrease (Bezold-Jarisch reflex). 2,3-butylene glycol dilatates the capillary vessels of the frog's web.

Discussion. In patients with uremia or hepatic coma, blood pyruvic acid is elevated. In men pyruvic acid is converted to acetyl-coenzyme A, or to acetoin and 2,3-butylene glycol. In patients with renal and hepatic disease, the conversion of pyruvic acid to acetyl-coenzyme A might be markedly reduced and the formation of acetoin from pyruvic acid increased. Increased acetoin and 2,3-butylene glycol in blood could be demonstrated in patients with renal or hepatic disease[10,11].

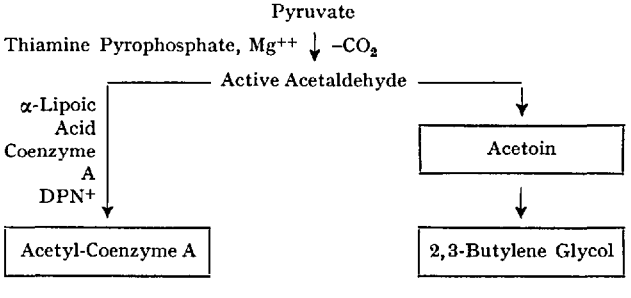

Fig. 1. Metabolism of pyruvic acid in men and mammals.

[1] The study was aided by grants from 'Schweizerischer Nationalfonds' and from F. Hoffmann-La Roche & Co. AG, Basel.

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