Table II. Turbidimetric assay of hyaluronidase activity in extracts of mucus glands and testes of the drone

	Protein (μg)	Hyaluronidase activity (% fall in turbidity)								
		Hyaluronic acid				Chondroitin-4-sulfate				
Time (h)		1	2	3	8	1	2	3	8	22
Mucus glands	47	0	10	31	80	2	5	7	20	45
Testes	187	0	0	14	37	6	6	6	13	28

The assay system consisted of 0.5 ml high speed homogenate supernatant and 0.5 ml substrate solution containing 200 μg hyaluronic acid or chondroitin-4-sulfate in 0.1 *M* acetate buffer – 0.15 *M* NaCl pH 5.0. After incubation at 37 °C 2 ml of a 2.5% solution of cetyltrimethylammonium bromide in 0.5 *M* NaOH were added and the turbidity developing was recorded at 400 nm. The fall in turbidity is expressed as a percentage of the turbidity developing in the intact control substrate.

Experiments consisting of the examination of the effects of pH and temperature showed similar characteristics of the enzymes of the 2 insect sources. Thus, pH profiles and optima were identical (4.5 with both hyaluronic acid and chondroitin-6-sulfate as substrates) as were also the profiles of enzymatic activities in relation to incubation temperatures (Figures 1 and 2). Of significance is the increase in activity from 37 °C to 56 °C, which is in contrast with the behavior of mammalian testicular hyaluronidase. The latter is gradually inactivated in this range[1]. This observation led us to examine the stability to heating of the insect hyaluronidase. Such experiments showed that it was almost unaffected by heating for at least 3 h at 50 °C, a temperature which promptly causes inactivation of mammalian testicular hyaluronidase[1,6]. Further work will be required in order to see whether this stability is a characteristic of the enzyme or is due to the presence of stabilizing factors in the crude extracts investigated.

The identical behavior of the hyaluronidases of accessory glands and testes of the drone, with respect to the parameters examined, raises the question whether they have a common origin. It would be difficult to assume, however, that the testicular tissue or the spermatozoa are the sources, since the accessory glands exhibited a higher specific activity than the testes.

Although it is generally supposed that in mammals the spermatozoal hyaluronidase is involved in the process of insemination, its precise role is still uncertain. The presence of hyaluronidase in the mucus accessory glands of the drone might be associated with the special features of the fertilization process in the bee. Thus, when drone and queen bee meet, the male, by explosive contraction of the abdomen, everts part of its reproductive system including spermatozoa and mucus into the genital tract of the bee. After each mating (multiple mating is common practice with the honey bee) the spermatozoa are forced out of the oviducts into the spermatheca where they are stored for very long periods of time (2 to 4 years). This latter process involves, in an interval of not more than 24 h after mating, the passage of spermatozoa through a very narrow channel, the spermathecal duct, whose particular structure permits control by the queen of the discharge of only small numbers of spermatozoa at the time of the discharge of eggs[7]. Possibly because of the resistance encountered during the flow through the narrow duct, only about 10% of the supply of spermatozoa received by the queen reach the spermatheca, the remainder being disposed off. The mechanism enabling the entry of a relatively large number of spermatozoa into the spermatheca is not clear. We suppose that the mucus of the male accessory glands might serve as the source of an enzyme capable of depolymerizing the mucopolysaccharides of the spermathecal duct, thus facilitating the flow of the spermatozoa to their site of storage. Further experiments are now in progress to test this hypothesis.

Zusammenfassung. Es wird in Hoden und akzessorischen Schleimdrüsen der Drohne das Vorkommen einer Hyaluronidaseaktivität nachgewiesen, die durch die Spaltung von Hyaluronsäure, Chondroitin-4- und -6-sulfat charakterisiert ist und eine bedeutend höhere Temperaturresistenz im Gewebeextrakt als die vergleichbaren Enzyme aus Säugergeweben zeigt.

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Arginine-rich Low Molecular Peptides in Human Neoplastic Serum

It has previously been found that, in the serum of patients with malignant neoplasms, the arginine content of the albumin fraction is increased[1]. These changes have not been observed in pathological conditions not due to neoplasma but with an increased tissue decomposition[2]. An increase in arginine content has also been observed in fibrin formed during the clotting of fibrinogen with thrombin from persons suffering from neoplasms[3]. It would therefore seem that in cases of neoplastic diseases, low molecular substances appear in the blood. These substances are probably peptides, either bound by serum

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Total arginine and free arginine in human neoplastic blood sera

Group studies	Total arginine (μM/l of serum)	Free arginine (μM/l of serum)	Peptide bound arginine (μM/l of serum)
Neoplastic serum	143.9 ± 14.6	78.8 ± 12.9	65.1 [a]
Control serum	82.7 ± 12.7	80.8 ± 11.2	lack or trace [b]

[a] Calculated from total and free arginine. [b] In most chromatograms the colour spots on arginine were not obtained.

albumin or incorporated into the fibrin clot. In the investigations presented here, the low molecular substances in the serum of persons suffering from neoplasma, giving a ninhydrin reaction, were analyzed chromatographically in order to find specific peptides rich in arginine.

Material and methods. 15 sera from person suffering from neoplasms and 10 control sera were analyzed. Among the cases of malignancies, there were 5 lung neoplasms, 7 gastrointestinal cancers and 3 neoplasms of the rectum. The sera were deproteinized with methanol and acetone (2:1), degreased with chloroform and concentrated by evaporation. The total arginine content was then determined by the Sakaguchi method[4]. The material was analyzed by means of ascending paper chromatography in the following system: butanol, acetic acid, water, in a ratio of 4:1:1. The spots corresponding to free arginine were eluted and their arginine content was determined by the same method[4].

The peptide spots were eluted with water, hydrolized in 6 *N* HCl and separated chromatographically in the abovementioned system. In addition, the staining test for arginine was performed on the paper.

Results and discussion. The total arginine content of the sera from persons suffering from neoplasms was found to be 143.9 μM/l, that of the healthy persons 82.7 μM/l. The content of free arginine in both investigated groups was the same: 78.8 μM/l and 80 μM/l. The total increase of arginine in the neoplastic sera was 74% and consisted only of peptide-bound arginine (Table).

The composition of the free amino acids in the serum of persons with neoplasms is identical with that of the serum of healthy persons (Figure 1), except for slight differences

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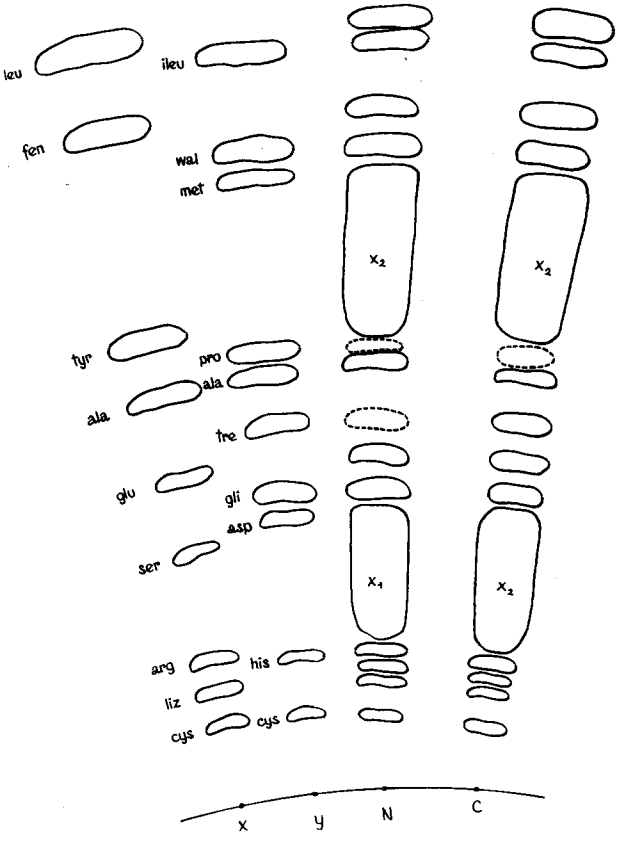

Fig. 1. The spots of amino acids and peptides of the control and neoplasms sera. X and Y, samples spots; C, control sera; N, neoplastic sera; X_1 and X_2, peptides spots.

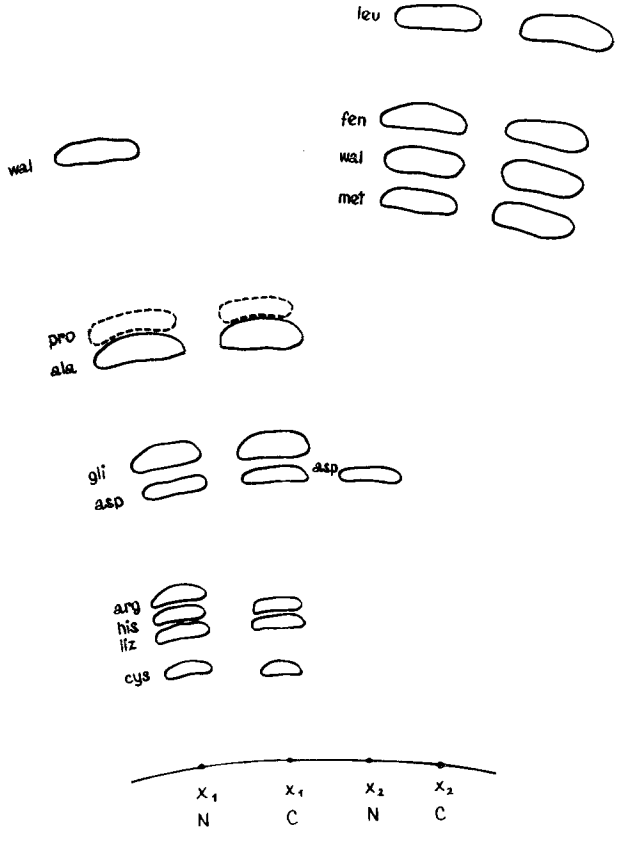

Fig. 2. The spots of amino acids after hydrolysis of X_1 and X_2 peptides. $\frac{X_1C}{X_2C}$ spots of amin acids from control sera; $\frac{X_1N}{X_2N}$ spots of amino acids from neoplastic sera.

in the intensity of the ninhydrin-positively stained spots. The peptides occurring in both types of sera have a different aminoacid composition. The peptides designated X_1 are characterized by the presence of basic amino acids, and in the neoplastic sera by an additional content of arginine and valine (Figure 2). The X_2 peptides from the control sera contained leu, val, met, phen amino acids. The X_2 peptides from neoplastic sera contained aspartic acid in addition to the other amino acids.

The investigations showed that in the total arginine content of the sera from persons with neoplasms is comprised of free L-arginine in an amount corresponding to that of the sera from healthy persons and a peptide-bound arginine which was not found in the control sera. In neoplastic diseases, therefore, peptides characterized by the presence of the amino acid arginine appear in the blood serum. SKARZYNSKI and SARNECKA-KELLER[5] were the first to suggest that in certain pathological conditions, for example malignancy, and in tissue damaged by radiation, compounds not found in healthy person's sera may be found in the sera of such patients. HAMMERSTEN and SANDELL[6] in investigations on sera from leukaemia patients, isolated a peptide with an amino acid content similar to that found in our investigations, which always contained arginine and besides serine and methionine.

It is interesting to note that, in all the sera from the patients with neoplasms, no matter what the localization of the tumor, and identical amino acid composition was found in the X_1N peptides. It is suggested that these peptides may have originated from disintegrated tumor basic proteins. Such proteins in human tissue tumors[7] and in an experimental by induced Guerin tumor in rats[8] has been found recently.

It is thought that, under the influence of specific intracellular proteases and endopeptidases, the proteins are degraded into peptides. As a result of disturbances in the permeability of the cell membranes, the protein degradation products may escape into the extracellular spaces and thus into the blood stream.

Zusammenfassung. Nachweis von argininreichen Peptiden in menschlichen Seren mit neoplasmatischen Krankheiten.

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Inhibitory Effects of Nitrososarcosine on Mouse Liver Mixed Function Oxidase Activity

Potential human hazards from environmental exposure to dialkylnitrosamines have become a matter of major concern[1,2]. That humans are sensitive to the toxic effects of nitrosamines is shown by observations that human liver contains enzymes which can activate dimethylnitrosamine[3]. Nitrosamines are rather inert, chemically, and derive biological action from enzymatic activation to carbonium ions[4]. The organ distribution of enzymes which activate nitrosamines generally determines the organotropic action of the various nitrosamines[5].

Nitrososarcosine induces cancer of esophagus in rats[6] and squamous cell carcinoma of the nose in mice[7]. The 7-day oral LD_{50} in mice is 3.15 g/kg with little noticeable histological changes. Therefore, since there is no acute liver pathology, it seems unlikely that enzymes necessary to activate nitrososarcosine would be present in mouse liver.

It is the purpose of the present communication to report biochemical effects of nitrososarcosine on mouse liver-namely, inhibition of microsomal enzyme activity. The significance of this phenomenon is that it is one of the only biological effects of a diakylnitrosamine unassociated with apparent enzymatic activation.

Male Swiss albino mice (ICR/dub) were used in all studies. Animals were housed in shoebox type cages with constant access to Purina Chow and water. Mice were killed by cervical dislocation and mouse liver microsomal enzyme function was assessed by quantitating aminopyrine demethylase and aniline hydroxylase activity. Microsomal suspensions were prepared as described previously[8].

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Table I. Dose response of mouse liver aminopyrine demethylase and aniline hydroxylase activity to nitrososarcosine

Treatment	No. of animals	Aminopyrine demethylase activity[a]	Aniline hydroxylase activity[a]
		(Mean ± S.E.)	(Mean ± S.E.) × 10
Control	10	3.75 ± 0.39	7.81 ± 0.09
Nitrososarcosine (250 mg/kg)	10	2.74 ± 0.32[b]	6.42 ± 0.03
Nitrososarcosine (500 mg/kg)	10	1.89 ± 0.24[c]	5.30 ± 0.06[c]
Nitrososarcosine (1000 mg/kg)	10	1.52 ± 0.10[c]	2.98 ± 0.03[c]

[a] Expressed as mMoles of product per g liver per 10 min. [b] Statistically different from controls at $p < 0.05$. [c] Statistically different from controls at $p < 0.01$. Groups of mice were treated with nitrososarcosine, p.o., and sacrificed 3 h later.