controls were obtained from the autopsies of non-cancer patients. The tissue was first homogenized in 0.1 *N* sulfuric acid (1:7 w/v) with a Waring Blendor, and then the acid homogenate was heated at 80 °C for 1 h in order to liberate the ketosidically bonded sialic acids[6]. After removal of the sulfuric acid as insoluble barium sulfate, the hydrolysate plus 3 water washings of the sediment were passed successively through volumns (1 × 23 cm) of Dowex 50 W (H^+) and Dowex I (formate). The columns were washed with distilled water, and the sialic acid was eluted from the Dowex I (formate) column with 0.3 *N* formic acid. The effluent was collected in 10-ml fractions with an automatic fraction collector. All sialic acid positive fractions were pooled and lyophilized to remove formic acid, and the residue was dissolved in distilled water. The sialic acid content was then determined by the thiobarbituric acid method[7] with crystalline NANA as the standard. *Identification* of NANA and NGNA was accomplished by paper chromatography[8] on Whatman No. 1 Chromatography paper using *n*-butanol-*n*-propanol-0.1 *N* HCl (1:2:1) and the sialic acids were detected with the thiobarbituric acid spray-reagent[9]. A mixture of standard NANA and NGNA was run on the same paper, next to the sialic acid isolated from tissues.

Results and discussion. The chromatographic analyses of the sialic acids isolated from malignancies found in 4 different organs – pancreas (adenocarcinoma), liver (metastasis from pancreatic adenocarcinoma), skin (squamous cell carcinoma) and paratracheal lymph node (metastasis from skin melanoma), revealed the presence of NANA but no evidence for the presence of NGNA was ever obtained.

The sialic acid content of the cancerous tissues was considerably elevated. The values obtained in the analyses of pancreatic tissues (expressed as mg of NANA per 100 g of fresh tissue) were 45.8 for normal pancreas, 187.0 for the adenocarcinoma and 75.5 for the liver metastasis of pancreatic adenocarcinoma.

The results of the experiments described indicate that, unlike the situation observed in the rat, both normal and cancerous human tissues contain only NANA.

Our quantitative studies support previous findings of BARKER et al.[2], since the cancerous tissues of pancreatic origin were shown to contain from 2 to 4 times as much NANA as the normal control. The cause of this sialic acid increase and its functional significance in malignancy have yet to be determined. Studies of NANA concentration and of the distribution of the various macromolecular species containing NANA in subcellular fractions should help to elucidate this problem[10].

Zusammenfassung. Die Sialinsäure welche im menschlichen Krebsgewebe vorhanden ist, wurde isoliert, charakterisiert und gemessen. Im krebsartigen und normalen Gewebe wurde nur N-Acetylneuraminsäure gefunden. Die Sialinsäure im Pankreas-Adenokarzinom war ums 4fache grösser als in normalen Kontrollgeweben.

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[6] L. SVENNERHOLM, Acta chem. scand. *12*, 547 (1958).
[7] L. WARREN, J. biol. Chem. *234*, 1971 (1959).
[8] E. SVENNERHOLM and L. SVENNERHOLM, Nature, Lond. *181*, 1154 (1958).
[9] L. WARREN, Nature, Lond. *186*, 237 (1960).
[10] This investigation was supported in part by U.S. Public Health Service Grants No. AI-04954 and No. NS-09176 from the National Institutes of Health. E.W.M. acknowledges a Medical Student Fellowship awarded by the Council for Tobacco Research. R.C. is a Research Career Development Awardee (No. 5-K3-AM-38649) of the National Institute of Arthritis and Metabolic Diseases.

The Effect of Surgical Trauma on Rat Secretory Neurons

Sufficient evidence elucidating the characteristic morphophysiological changes in the mammalian neurosecretory system in response to traumatic stress has now accumulated in the literature. The stressful procedures employed to induce such responses are extremely varied and range from pin pricks on rats' tails[1] to severe thermal burns on the dorsal skin of guinea pigs[2]. However, the effects of planned surgery – the commonest clinical event – on the secretory system have largely escaped attention, despite the fact that the complex regulation of post-operative fluid dynamics is almost entirely governed by the secretory axis. Consequently, the effects of surgical stress were investigated and the present report describes the structural and secretory changes in the rodent neurosecretory system following an operative trauma of moderate intensity. The morphological effects of a more severe surgical stress (spinal transection) were reported earlier[3,4].

Materials and methods. 20 male Wistar rats (250–300 g) on water ad libitum were used. Of these, 16 animals were subjected to a 3″ linear incision on their tails (the incision being carried through the skin and subcutaneous tissue upto the caudal vertebrae) under i.p. Nembutal anaesthesia (6 mg/10 g wt.) and were killed under chloroform at 8 h intervals in groups of 2. Two each of the 4 control rats were sacrificed under chloroform at 0 and 16 h respectively following initial anaesthetic doses of Nembutal.

Half the total number of operated and control animals from each group were used for cytological and cytometric studies. For this purpose, paraffin sections of gluteraldehyde-perfused material (2.5% gluteraldehyde solution buffered to pH 7.3 with 0.067 *M* cacodylate, containing 0.9% sodium chloride) of brains and pituitary glands were stained with 1% cresyl fast violet acetate. Cytometry was carried out on the supraoptic (SON) and paraventricular (PVN) nuclei with a micrometer eye-piece and the data were processed and analyzed in a computer.

The remaining animals were used for the study of neurosecretory substance (NSS). Alternate paraffin sections of formalin-fixed material were stained with chrome-alumhaematoxylin phloxin (CAHP)[5] and per-

[1] A. B. ROTHBALLER, Anat. Rec. *115*, 21 (1953).
[2] K. MIETKIEWSKI, A. PISKORZ, F. KOPACZYK and J. HRYNIEWIECKI, Endokr. pol. *18*, 133 (1967).
[3] S. R. CHOUDHURY, J. Anat. *106*, 175 (1970).
[4] S. R. CHOUDHURY, Acta Anat. *79*, 84 (1971).
[5] W. BARGMANN, Z. Zellforsch. *34*, 610 (1949).

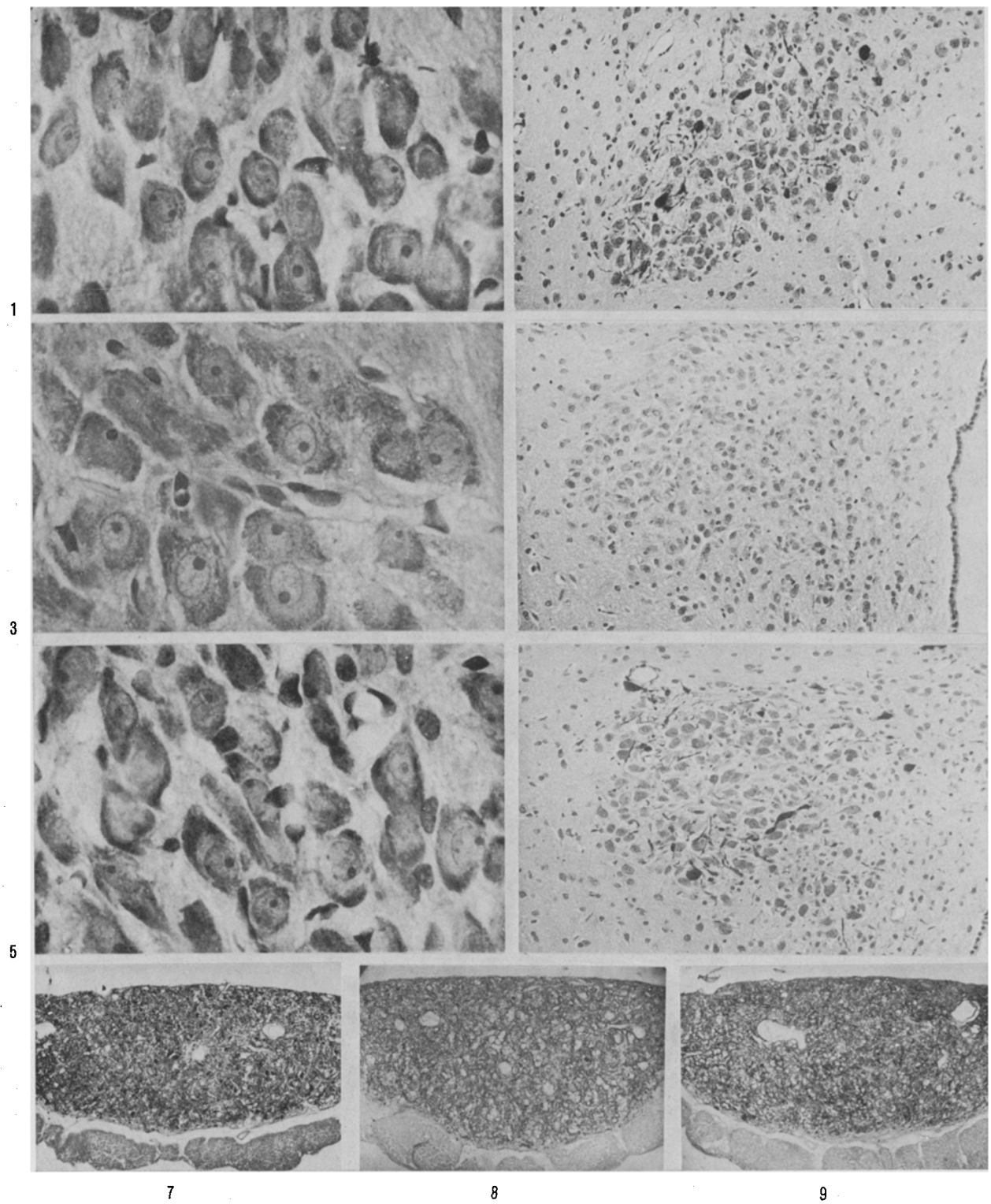

Fig. 1. SON in control rat showing typical resting features, 16 h after Nembutal administration. Cresyl fast violet acetate, ×451.
Fig. 2. PVN in control rat showing presence of NSS, 16 h after Nembutal administration. CAHP, ×112.
Fig. 3. SON, 16 h after linear incision. Enlargement of all neuronal components and other features of stress (see text) are evident. Cresyl fast violet acetate. ×451.
Fig. 4. PVN, 16 h after operation. The nuclear area is devoid of NSS. CAHP, ×112.
Fig. 5. SON, 32 h after operation. The cytology resembles control pattern. Cresyl fast violet acetate. ×451.
Fig. 6. PVN, 24 h after operation. Reaccumulation of a certain amount of NSS (predominantly intraLaxonal) is noticeable. CAHP, ×112.
Fig. 7. Neurohypophysis in control rat, 16 h after Nembutal anaesthesia. A moderate amount of NSS is seen dispersed throughout the parenchyma. PAB, ×44.
Fig. 8. Neurohypophysis, 16 h after operation. A certain amount of NSS is seen in this section. The picture, however, may be variable (see text). PAB, ×44.
Fig. 9. Neurohypophysis, 32 h after operation. Stainable material can be seen dispersed throughout the parenchyma. PAB, ×44.

Results of linear incision

Time interval after operation or anaesthesia (h)	Supraoptic			Paraventricular		
	Cyton	Nucleus	Nucleolus	Cyton	Nucleus	Nucleolus
Control						
16 h after Nembutal	162.08±5.57	43.60±0.90	5.07±0.18	155.78±5.14	40.98±1.31	4.23±0.17
8	174.67±4.70 0.10–0.05	52.96±1.52 <0.001	6.97±0.28 <0.001	167.85±4.52 0.10–0.05	55.67±1.57 <0.001	7.36±0.28 <0.001
16	224.40±5.78 <0.001	84.39±2.82 <0.001	9.75±1.84 0.02–0.01	202.28±5.80 <0.001	58.37±2.27 <0.001	8.28±1.33 0.05–0.001
24	210.00±4.22 <0.001	64.83±2.99 <0.001	7.69±1.29 0.05–0.025	206.36±50.09 <0.001	62.37±2.79 <0.001	6.23±0.17 <0.001
32	183.72±5.54 0.01–0.005	51.55±1.89 <0.001	7.18±0.33 <0.001	181.02±4.54 <0.001	54.13±1.29 <0.001	6.84±1.28 0.05–0.025

The figures represent cross-sectional areas in square microns with their standard errors. The significance of the difference in means from the control values are shown in each case. Values of $2P<0.05$ are significant.

formic acid-alcian blue (PAB)[6] and every 3rd section with cresyl fast violet acetate. In all cases, neighbouring hypothalamic neurons were also examined to detect any changes occuring due to fixation or operative procedures.

Results. The neurons in SON and PVN of control animals showed large nuclei and prominent nucleoli and contained abundant chromidial material (Figure 1). A certain amount of NSS was present in both these nuclear areas and also in the neurohypophyses (Figures 2 and 7). Neurons with these features are subsequently denoted as 'resting' neurons.

Following operation, little deviation from the control pattern was noted upto 8 h. During the subsequent period SON and PVN underwent progressive enlargement, reaching a peak at 16 h. Frequent nuclear eccentricity and marginal displacement of the chromidial substance were noted at this stage (Figure 3). The secretory nuclear areas were also devoid of NSS (Figure 4). Neurons with such characteristic appearances are referred to as 'stressed' for descriptive convenience. Features of stress persisted at 24 h, although the first reaccumulation of NSS was observed at the end of this period (Figure 6). During the next 8 h a further retrogression in neuronal dimensions was noted (Figure 5) and the secretory features reverted to the basal resting pattern at this stage.

The neurohypophyses in all operated cases exhibited wide variations in their secretory contents, but were never totally devoid of the latter at any stage (Figures 8 and 9). The amount of NSS did not appear to bear any definite relationship with the post-operative interval. The cytometric results are tabulated in the Table.

Discussion. With certain reservations the enlargement in the hypothalamic secretory neurons coupled with the disappearance of NSS have been regarded as manifestations of augmented neurosecretory activity[7,8]. Based on such indices, the present findings suggest that an operative procedure of moderate intensity induces progressive hypothalamic activation. Following an initial period of inertia the secretory neurons are gradually stirred into activity, reaching a peak around 16 h. The subsequent period is marked by neuronal retrogression with reaccumulation of NSS – features which indicate a return towards the resting functional state.

From the above response pattern it appears that the hypothalamic behaviour follows a well-defined and regular sequence. The neurohypophysial secretory contents, however, vary greatly and apparently bear no relationship to activities in the nuclear region. Being in a continual state of flux, the neurohypophysial NSS probably represents the more labile and readily available component in this bi-compartmental secretory axis. Confirmation of the above hypothesis has also been obtained from more elaborate studies[9]. The source of neurohypophysial NSS during its absence from the hypothalamic areas (around 16 h following trauma) is not clear. It is possible that the precursor of hormone molecules are being actively synthesized in the nuclear areas to be transported to the neurohypophysis in submicroscopic form. Alternatively, the hypothesis of hormone biosynthesis occuring along the entire length of the secretory axis as postulated, among others, by SACHS[10], is not inconsistent with the present observations.

The present cytometric results (Table) indicate that the overall response of SON and PVN to the linear incision procedure is similar. However, employing a more severe traumatic procedure, CHOUDHURY[4] earlier reported a differential response between the secretory nuclei (activation in SON being of longer duration and of greater amplitude compared to those in PVN). The significance of the present finding is difficult to interpret since the current concept of secretory activity supports the existence of a functional difference between SON and PVN[11].

The increased activity following operative stress is most likely due to an attempt to snythesise and elaborate more neurohypophysial hormones, particularly ADH, since antidiuresis is an inevitable response to any form of trauma. This is indirectly, but strongly corroborated by the findings of other workers of an increased plasma ADH activity following painful stimulation[12,13]. The nature of

[6] C. W. M. ADAMS and J. SLOPER, J. Endocr. *13*, 221 (1956).
[7] R. ORTMANN, in *Handbook of Physiology* (Eds. J. FIELD and H. W. MAGOUN; American Physiological Society, Washington (1960), vol. 2, p. 1039.
[8] J. C. SLOPER and B. C. KING, J. Path. Bact. *86*, 179 (1963).
[9] S. R. CHOUDHURY, Acta Anat., *80*, 136 (1971).
[10] H. SACHS, J. Neurochem. *5*, 297 (1960).
[11] A. B. ROTHBALLER, in *Endocrines and the Central Nervous System* (Ed. R. LEVINE; William and Wilkins Co., Baltimore 1966), p. 86.
[12] I. A. MIRSKY, M. STEIN and G. PAULISH, Endocrinology *54*, 491 (1954).
[13] W. H. MORAN, F. W. MILTENBERGER, W. A. SHUAYB and B. ZIMMERMAN, Surgery *56*, 99 (1964).

causal stimuli for hypothalamic activation appear to be complex and multifactorial. Mental stress, effects of anaesthesia, afferent impulses from the operative site (pain in particular), blood loss, noxious products of cell damage and any unappreciated variations in post-operative fluid uptake – all are contributory. Amongst these, hurtful afferent discharges from the operative site appear to be most dominant. This postulation is supported by denervation experiments which will be reported separately[14].

Zusammenfassung. Es werden Zellveränderungen des Hypothalamus bei traumatischen Hautschädigungen untersucht und Vergrösserungen neurosekretorischer Zellen, Protoplasmaveränderungen sowie Veränderungen der Sekretionsprozesse festgestellt.

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[14] S. R. CHOUDHURY, in preparation.

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Protektive Wirkung einer Vorbehandlung mit Glukose gegenüber der Cyclophosphamid-Intoxikation bei weiblichen Mäusen

Die Frage der Toxizitätsminderung chemotherapeutisch verwendeter Alkylantien stellt ein aktuelles Problem dar, wenn es gelingt, die Giftigkeit einer alkylierenden Substanz ohne Abschwächung der erwünschten chemotherapeutischen Wirksamkeit zu vermindern. Arterenol[1], Serotoninantagonisten[2,3], Thiolverbindungen[4–7] als auch das Antihistaminikum Tripelenamin[8] vermindern die Toxizität verschiedener N-Lost-Derivate. Die vorliegende Untersuchung nahm ihren Ausgangspunkt von der Zufallsbeobachtung, dass weibliche Mäuse die i.v. Injektion von Cyclophosphamid (Endoxan) besser tolerierten, wenn als Lösungsmittel statt der üblichen 0,9%igen NaCl-Lösung eine 10%ige Glukose-Lösung verwendet wurde.

Als Versuchstiere dienten NMRI-Mäuse (SPF-Koloniezucht, Süddeutsche Versuchstierfarm, Tuttlingen) mit einem Körpergewicht von 28–31 g. Cyclophosphamid kam als NaCl-freie Substanz zur Anwendung. Aus Vorversuchen ergab sich, dass bei prophylaktischer Gabe von 2000 mg/kg Glukose s.c. eine gesteigerte Toleranz gegenüber der Cyclophosphamid-Intoxikation auftrat. Der optimale Zeitabstand zwischen der prophylaktischen s.c. Glukoseinjektion und der s.c. Gabe von Cyclophosphamid zeigte eine Abhängigkeit von der Raumtemperatur. Bei einer Raumtemperatur von 23 °C erwiesen sich 2 h, bei einer Raumtemperatur von 26 °C 1 h als Zeitintervall zwischen Glukose- und Cyclophosphamidapplikation als optimal.

Aus der Abbildung geht hervor, dass die prophylaktische Gabe von 2000 mg/kg Glukose die DL_{90} von Cyclophosphamid weitgehend verringern kann. Hingegen erhöht die stereoisomere Verbindung der Glukose, die Fruktose, die Toleranz gegenüber der DL_{90} von Cyclophosphamid nicht. Eine gewisse Beschleunigung der Absterberate lässt sich feststellen, wenn 0,6 ml physiologische Kochsalzlösung eine Stunde vor der DL_{90} von Cyclophosphamid gegeben wurde. Versuche an männlichen NMRI-Mäusen zeigten, dass die Toleranz gegenüber der Cyclophosphamid-Intoxikation durch Glukose weniger deutlich beeinflusst wird. Hingegen kommt es bei kastrierten männlichen Mäusen, insbesondere bei männlichen Mäusen, welche über 8 Tage 0,1 mg/kg Diäthylstilboestrolpropionat subcutan erhielten, zu Ergebnissen, welche mit den in der Abbildung dargestellten weitgehend übereinstimmten. Die Frage des Wirkungsmechanismus der Toleranzerhöhung durch Glukose, welche eine deutliche Hormonabhängigkeit aufweist, bleibt offen.

Summary. Pretreatment of female mice by 2000 mg/kg glucose 1–2 h before the application of the DL_{90} of cyclophosphamide induced a remarkable protective effect. A similar increased tolerance by glucose pretreatment in male mice succeeded only in castrated or diethylstilboestrol treated animals.

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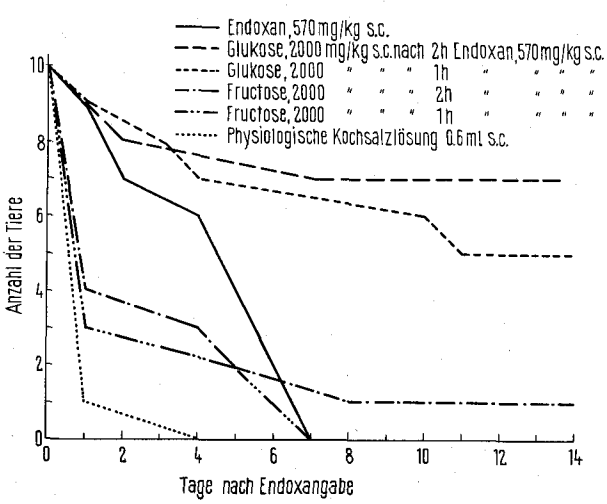

Absterbekurve weiblicher NMRI-Mäuse unter dem Einfluss der DL_{90} von Cyclophosphamid (Endoxan) bei prophylaktischer Gabe von Glukose, Fructose oder physiologischer Kochsalzlösung.

[1] J. B. FIELD, Proc. Am. Ass. Cancer Res. *3*, 224 (1961).
[2] G. BALLERIMI und L. BOSSI, Experientia *21*, 334 (1965).
[3] J. B. FIELD, E. C. DOLENDO, A. MIRELESS und B. H. ERSHOFF, Cancer Res. *25*, 382 (1965).
[4] H. BRINCKER, Acta path. microbiol. scand. *61*, 52 (1964).
[5] T. A. CONNORS, Europ. J. Cancer *2*, 293 (1966).
[6] S. P. IARMONENKO, V. M. BERGOLT'S, R. G. ALIEV, A. V. KIL'DISHEVA, M. G. LINKOVA und J. L. KUNIANTS, Dokl. Akad. Nauk SSR *126*, 476 (1965).
[7] L. LARIONOV und J. SPASSKAYA, IX. Int. Cancer Congress Abstract (1966), p. 361.
[8] D. B. ROCHLIN, H. GOEPFERT und J. DAMNAVITS, Surgery *124*, 771 (1967).