Wie Farbwechselversuche mit der Sandgarnele (*Crangon crangon*) zeigten, ist der gefundene positiv myotrope Wirkstoff mit dem Farbwechselhormon der Crustaceen *nicht* identisch: Garnelen, die an weissen, schwarzen und mittelhellen Untergrund angepasst waren, wurden Eluate der myotrop wirksamen Zone 1 injiziert. Die Tiere zeigten keine Farbveränderungen.

Zum Vergleich wurden aus Augenstielextrakten von *Crangon* Chromatogramme unter denselben Bedingungen, wie für *Astacus* beschrieben, entwickelt. Diese Chromatogramme sind denjenigen von *Astacus* gleich oder zumindest sehr ähnlich. Sie färben sich mit denselben Reagenzien in gleicher Weise an, und die Substanzen, die bei *Astacus* nicht nachweisbar waren, wurden hier ebenfalls nicht gefunden. Auch die R_F-Werte der einzelnen Zonen scheinen bei beiden Arten übereinzustimmen. Jedoch ist die Anzahl der Versuche, die mit *Crangon* durchgeführt werden konnten, zu gering, um hier bindende Aussagen zu machen.

Um einen weiteren Einblick in die chemische Natur des Wirkstoffes zu gewinnen, wurden Eluate der Zone 1 48 h in 5*n* Salzsäure gekocht und dann in einem 2dimensionalen Chromatogramm entwickelt. Es wurde hierbei nach der Methode von ZAHN[3] vorgegangen. Dieses 2dimensionale Chromatogramm zeigte 6 Flecke, deren chemische Konstitution noch nicht näher bestimmt wurde. Jedoch geht aus diesen Versuchen hervor, dass der myotrop wirksame Bestandteil des Augenstielextraktes eine Verbindung ist, die aus mehreren Aminosäuren besteht. Es handelt sich also höchstwahrscheinlich um ein Peptid.

Eine ausführliche Besprechung der Versuche und ihrer Ergebnisse wird an anderer Stelle erfolgen.

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Summary

The detection of two myotropic substances in the eyestalk and the brain of the crayfish *Astacus astacus* and the shrimp *Crangon crangon* was described[2]. Both substances have an action on the isolated ileum of the guinea pig. The substance extracted from the eyestalk has a positive myotropic one, the substance extracted from the brain an inhibiting one.

Further a method is given for the separation and purification of the positive myotropic substance of the eyestalk by paper-chromatography.

Primary Cancer of the Liver in Rats after Subcutaneous Implantation of Pellets of Progesterone and Testosterone Propionate

The object of this communication is to report the development of primary hepatocellular carcinoma of the liver in castrated female rats implanted subcutaneously with pellets of testosterone propionate and progesterone.

During a recent investigation into the relation of diet to the incidence of spontaneous tumours in our strain of rat, the ovaries of aged female rats, receiving a diet which increased significantly the frequency of uterine cancers, often contained large corpora lutea, the function of which was not reflected by the state of the vagina or uterus. In view of the presence of a large interstitial body on the ovary, together with the corpora lutea, it was suspected that the uterine and vaginal reactions were the consequence in part of the combined effects of variable amounts of progesterone and of androgen. The possibility was therefore entertained that prolonged stimulation with progesterone and testosterone might in some way be causally related to the increased incidence of uterine cancer observed in our experimental rats. Accordingly, this possibility was tested experimentally.

A group of 11, six-week old female rats, were castrated and immediately thereafter implanted subcutaneously with two pellets, one of progesterone (8 to 10 mg) and one of testosterone propionate (8 to 10 mg). The pellets were prepared in a special mould from the pure, crystalline hormones. As soon as palpation revealed a marked decrease in the size of either pellet, a further pellet was implanted while the rat was under ether anaesthesia. These rats received the same ration as fed our stock animals[1].

Six of the rats died from various causes between the 112th and 543 rd days of the experiment. Apart from reproducing the uterine and vaginal pictures observed in the rats used for the dietary experiments mentioned above, no noteworthy reactions were detected.

In one of the five surviving rats a greatly enlarged liver was palpated on the 696th day after the implantation of the pellets. The animal was killed and a large tumour was found in the left median lobe with multiple secondary nodules in the other lobes. Histological examination disclosed the tumour to be a primary liver cancer of the hepatocellular, trabecular variety. The main portal vein in the hilum contained masses of tumour cells and this was apparently responsible for widespread intrahepatic dissemination. Palpation of the abdomen of the 4 remaining rats disclosed the presence of another liver tumour in one rat which proved on microscopic examination also to be an hepatocellular carcinoma. Macroscopically, this tumour was 2 cm in diameter and was confined to the left lateral lobe. The rest of the liver appeared normal. No tumours were found at laparotomy in the other 3 rats, which are still alive.

The occurrence of two cases of primary liver cancer in this small series of rats is highly significant for the following reasons: (a) CURTIS and BULLOCK[2] found only 1 benign 'hepatoma' in 2450 rats surveyed during their study of tumours; (b) in 1954 we[3] reported our failure to produce liver cancer by methods other than those requiring the use of carcinogens, despite the fact that we had induced many varieties of liver pathology in several hundreds of rats; (c) in a special study of spontaneously-occurring tumours in approximately 1500 rats in our colony we did not find a single case of primary liver cancer; (d) GUERIN[4] commented on the absence of primary liver cancer in 16,500 rats investigated.

The liver tumours developing in our hormone treated rats were not preceded by cholangio-fibrosis, necrosis or cirrhosis. However, fibrosis did occur between and around the large cancerous nodules. In the same way as fibrosis of the liver can be dissociated from an antecedent

[1] J. GILLMAN, C. GILBERT, and I. SPENCE, Cancer *6*, 494 (1953).
[2] F. D. CURTIS and M. R. BULLOCK, J. Cancer Res. *14*, 1 (1930).
[3] J. GILLMAN and C. GILBERT, Cancer *7*, 1109 (1954).
[4] M. GUERIN, D. C., *Tumeurs Spontanées des Animaux de Laboratoire* (Amédée Legrand 1954).

fatty change[5], from necrosis[6] and from the excessive accumulation of iron[7] so it would appear now that primary liver cancer, at least in the rat, can also be dissociated from observable necrosis, fibrosis and bile duct hyperplasia. In this connection it should be mentioned that LAWS *et al.*[8] found that, after feeding aceto-aminofluorene the neoplastic transformation in ZYMBAL'S gland arose from tubules which did not show any antecedent cystic or other pathological change, such as occurs commonly in the liver before the emergence of cancer. It may be too early to assert that the extensive liver disease, accompanied by cirrhosis, commonly seen in the African throughout the Continent of Africa may not necessarily predispose to liver cancer.

Since a malignant neoplasm arising from the epithelium of a respiratory bronchiole and different from that commonly seen in adenosis of the lung was also encountered in one of the rats affected also with liver cancer, it is not unlikely that progesterone and testosterone propionate may be able to induce carcinoma in organs other than the lung and the liver. If the findings can be confirmed in a larger series of rats now under investigation in our laboratory, a different orientation may be given to the attack on the etiology of primary carcinoma of the liver in man.

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Résumé

Le carcinome hépatocellulaire du type trabéculaire a paru dans 2 rats sur 5, 745 jours après l'implantation de boulettes de progestérone cristallin et de propionate de testostérone, pesant 8 à 10 mg chacune. Un de ces rats a développé un carcinome provenant de l'épithélium d'une bronchiole respiratoire. L'absence d'une cholangiofibrosite antécédente, d'une nécrose ou d'une cirrhose hors de l'endroit de la tumeur du foie chez les rats soumis aux épreuves expérimentales, peut nécessiter un nouvel examen de la signification des lésions hépatiques comme facteur prédisposant au cancer chez l'homme.

[5] J. GILLMAN and T. GILLMAN, *Perspectives in Human Malnutrition* (Grune and Stratton, 1951).

[6] J. GILLMAN, C. GILBERT, and I. SPENCE, Amer. J. Digest Dis. *19*, 211 (1952). – J. GILLMAN and C. GILBERT, Ann. N. Y. Acad. Sci. *57*, 737 (1954).

[7] J. GILLMAN and T. GILLMAN, *Perspectives in Human Malnutrition* (Grune and Stratton, 1951); Arch. Path. *40*, 239 (1945).

[8] J. O. LAWS, G. RUDALI, R. ROYER, and P. MABILLE, Cancer Res. *15*, 139 (1955).

From Parasympathomimetics to Parasympatholytics by Homologous Substitution

The effect of a drug on a biological object is the result of the interaction of drug molecules with receptors.

The activity of a drug is determined by the affinity and the intrinsic activity[1]. The affinity is the equilibrium constant for the interaction of the drug with the receptors, which is in the simplest case equal to the reciprocal of the dissociation constant of the drug-receptor complex. The intrinsic activity (i.a.) is the effect per unit of drug-receptor complex. Drugs with a high intrinsic activity on parasympathetic receptors are pure parasympathomimetics, those with an intrinsic activity zero, are competitive antagonists of the mimetics. Drugs with an intermediate intrinsic activity behave as parasympathomimetics or -lytics, depending on the state of the receptor system. Parasympathomimetics and -lytics have affinity to the same receptor system.

In a physico-chemical sense the reaction between drug and receptor implies an interaction between the electrical fields inherent to their charge-distributions. Not only the field inherent to the kationic and anionic sites and other regions with a high or low electron density, but also those which underly the Van der Waals forces and hydrogen bonds have to be taken into consideration. The affinity is coherent with the interaction between the fields in the most general sense. The intrinsic activity often will be connected with a special part of this interaction.

Two magnitudes are of special importance for the drug-receptor interaction and its biological effect: (a) the charge-distribution on drug and receptors, (b) the sterical configuration of drug and receptor as far as they interfere with the interaction between the fields. Little is known about the molecular properties of the receptors. Of the drug-molecules the chemical structure and certain physico-chemical properties are known.

The study of pharmacodynamics has to be based on the relation between the properties of the drug and the effect. On the basis of the results thus obtained conclusions may possibly be drawn about the properties of the receptors. Evidence is accumulating in the literature[2] that in homologous series of drugs a change in the structure is often combined with a gradual change in the affinity. In an analogous way the intrinsic activity may be expected to change gradually with the structure.

Most parasympathomimetics contain in their configuration a positive and a negative grouping, located on a certain distance[3].

The same holds for the -lytics. This indicates that these groupings are important for the affinity to the parasympathetic receptors. In contrast with the -mimetics, the -lytics have large groups on the 'negative' side of the molecule[4]. With respect to substitution on the positive side of cholinergic drugs, e.g. succinylcholine tested on the myoneural junction, it was found that successive replacing of methyl groups by ethyl groups resulted in a gradual change from mimetic to lytic[5]. On the basis of the considerations given before it may be expected that homologous substitution on the positive, R_3, or on the negative side, R', of the dioxolane compound 2249 F (dilvasène)[6], will result in a gradual change of the

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[3] C. C. PFEIFFER, Science *107*, 94 (1948). – H. R. ING, P. KORDIK, and D. P. WILLIAMS, Brit. J. Pharmacol. *7*, 103 (1952).

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