where no muscle fibers remain, the giant cells are imbedded in a region of fibroblasts. Such regions in the muscle tissue may be the site of origin for myoblastomas.

These responses in invertebrate tissues have particular significance, for they are reminiscent of changes seen in mammalian tissues and thereby support the concept that similar cell mechanisms occur in invertebrate pathology. The question, however, of whether invertebrate tumors are 'true' tumors remains an issue among cancer biologists.

*Zusammenfassung.* Verschiedenes, durch Röntgenbestrahlung hervorgerufenes Regenwurmneoplasma erweist sich den Vertebraten-Mioblastoma ähnlich. In solchen Tumoren werden zuweilen Riesenkerne gefunden. Bestrahlte Würmer weisen ebenfalls vielkernige Riesenzellen auf.

R. L. HANCOCK

*Hulls Cove (Maine USA), July 9, 1964.*

## Presence of a Slow-Contraction Inducing Material in Fluid Collected from the Rat Paw Oedema Induced by Serotonin

In previous studies on rat paw oedema we succeeded in demonstrating that the different inducting substances appeared to determine the time course of the oedema and also its responsiveness to various kinds of antagonistic drugs[1–3]. These findings suggested the occurrence of manifold processes in this phenomenon. We decided to study the mechanisms involved by searching for the presence of biologically active material(s) in the oedema fluid of the swollen rat paw. Since serotonin as inducer resulted in a larger degree of swelling than did most other inducing materials, which facilitated the collection of sufficient amounts of oedema fluid, we decided to commence the investigation with this kind of oedema.

Male albino rats (100–110 g) from our own colony were given a subplantar injection of 2 $\mu$g serotonin creatinine sulphate dissolved in 0.9% saline in both hind paws. The injected volume was 0.1 ml in each paw and control animals received a corresponding volume of saline. 30 min after the injection, at the time of maximal swelling, the rats were sacrificed by decapitation; both hind paws were cut off distal to the tarso-crural articulation and pressed between the rollers of a small mangle, specially constructed for this purpose. This procedure enabled us to collect 0.2–0.5 ml of fluid from the two hind paws of rats that were given the subplantar injection of serotonin, whereas five or more of the rats which had received saline were needed to yield the same amount of fluid. The fluid was slightly opalescent and faintly pinkish. After filtration through cotton, a clear fluid was obtained. The fluid was collected and preserved in a test tube that was immersed in ice-cold water. Biological activity was tested: (a) On the isolated uterus of rats that were given 0.01 mg of estradiol benzoate one day before removing the organ. The temperature of the isolated organ bath (2.5 ml) was kept at 30°C and Jalon's solution was used as organ bath medium. Serotonin creatinine sulphate, acetylcholine chloride and synthetic bradykinin[4] were used as reference compounds, and LSD-25, atropine sulphate and phenylbutazone served as antagonists. In order to prevent tachyphylaxis when serotonin was used, the administration of this substance was alternated with addition of acetylcholine. (b) In other experiments the isolated guinea-pig ileum was used. This organ was suspended in Tyrode solution in an organ bath having a volume of 2.5 ml and kept at 37°C. Histamine dihydrochloride was used as reference substance and phenbenzamine (Antergan) as specific antagonist. The use of the isolated guinea-pig ileum was necessary in view of the non-responsiveness of the rat uterus to histamine. Irrespective of the use of either of the isolated organs, the lever was loaded with 0.5–0.85 g and the contractions were recorded on a smoked drum. The oedema fluid was tested as soon as possible after collection from the paws.

When the isolated organs were exposed to fluid collected from paws of rats receiving a subplantar injection of saline, no contractions were observed with doses up to 0.3 ml. The fluid originating from the swollen paws of rats treated by subplantar administration of serotonin, induced marked contractions of the isolated organs when added in a volume of 0.1 to 0.3 ml to the organ bath. In Figure 1 a record is presented demonstrating characteristic responses of the isolated rat uterus to various materials. In this particular experiment the kymograph was run at a higher speed than usual, in order to obtain more detached information on the qualitative character of the responses. Acetylcholine induced instantaneous contraction, whereas with serotonin a short lag period preceded the response. The lag period was even more pronounced with bradykinin, furthermore with this substance the contraction occurred at a slower rate than with either acetylcholine or serotonine. When the uterus was challenged with the oedema fluid, the lag period and speed of

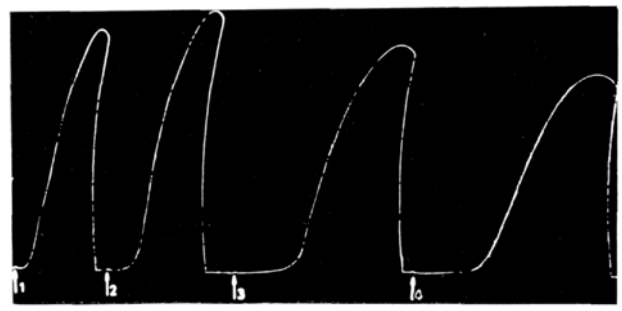


Fig. 1. Effect of various materials on the isolated uterus of the oestrus rat. 1 – Acetylcholine 0.75 $\mu$g. 2 – Serotonine 0.15 $\mu$g. 3 – Bradykinin (synthetic) 0.02 $\mu$g. 4 – Oedema fluid 0.1 ml.

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[4] Synthetic bradykinin was obtained through the courtesy of Prof. A. CERLETTI, Sandoz AG, Basel.

contraction were similar to the response obtained with bradykinin. Figure 2 shows that a dose of LSD-25 capable of blocking completely the contraction due to serotonin, induced no inhibitory effect on the response evoked by the oedema fluid. Under similar conditions no inhibition of the effect of the oedema fluid was obtained when atropine was added in a dose sufficient to abolish the response due to acetylcholine. Phenylbutazone, however, blocked the effect of both the oedema fluid and synthetic bradykinin, as shown in Figure 3. Furthermore, a partial inhibition but not a complete blockade of the response to the oedema fluid was obtained when phenbenzamine was used in a dose sufficient to block the effect of histamine completely.

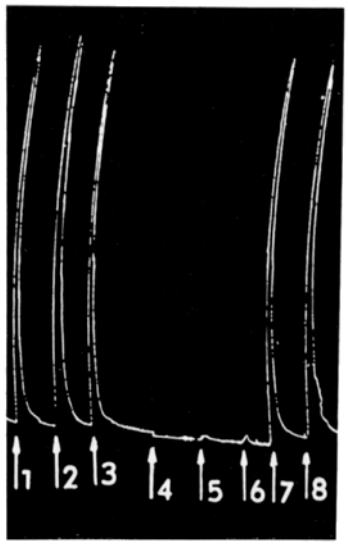


Fig. 2. Lack of blockade by LSD-25 of contraction evoked by oedema fluid on the isolated uterus of the oestrous rat. 1 – Serotonine 0.2 μg. 2 – Oedema fluid 0.2 ml. 3 – Acetylcholine 0.75 μg. 4 – LSD-25 5 μg. 5 – Washing. 6 – Serotonine 0.2 μg. 7 – Acetylcholine 0.75 μg. 8 – Oedema fluid 0.2 ml.

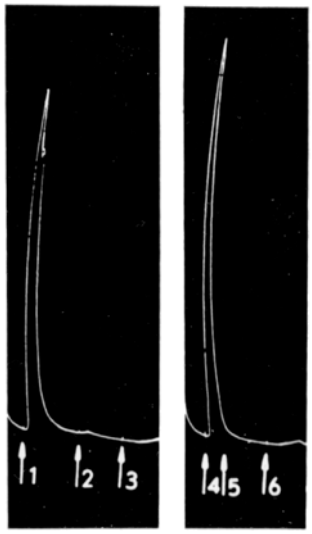


Fig. 3. Blockade by phenylbutazone of contractions evoked by oedema fluid and by synthetic bradykinin on the isolated uterus of the oestrous rat. 1 – Oedema fluid 0.15 ml. 2 – Phenylbutazone 1.25 mg. 3 – Oedema fluid 0.15 ml. 4 – Bradykinin 0.01 μg. 5 – Phenylbutazone 1.25 mg. 6 – Bradykinin 0.01 μg.

It should be remarked that the biological activity of the oedema fluid showed a tendency to decline even when preserved at 0°C. On the other hand, acid treatment of the fluid, followed by incubation at 37°C for 30 min and subsequent neutralization resulted in a marked enhancement of the biological activity, which could be abolished after addition of chymotrypsin.

The results presented indicate that the fluid collected from serotonine-induced rat-paw oedema displays marked smooth-muscle contracting properties. It appears, from the limited number of tests so far performed, that the biological activity is possibly due neither to acetylcholine nor to serotonin, but was partly caused by a histamine-like material and partly by a factor the properties of which resembled bradykinin and/or other related plasma kinins. It is pertinent to mention in this connection that the presence of a bradykinin-like material has been demonstrated in the perfusate of the 'thermic oedema (45°C)' in the rat's paw[5]. Our results suggest that the release of kinin-like substance(s) is not limited to the thermic oedema, but also occurred when oedema was induced by subplantar administration of serotonin. Presently we are studying fluids collected from other kinds of rat paw oedema, such as those induced by chicken egg white, kaolin, carrageenin, cobra venom etc. It has been shown that administration of phenylbutazone has no inhibitory influence on the development of the rat paw oedema induced by serotonin[3,5]. Phenylbutazone, however, markedly antagonized the smooth muscle contracting activity of the oedema fluid collected after serotonine-induced swelling of the rat paw. These two facts are not necessarily in contradiction with each other; firstly since it may be that the release of the kinin-like material is not a causative factor in the oedema development but merely a coincidental process or even a result of it, and secondly since phenylbutazone may antagonize some effects of plasma kinins whilst leaving other effects uninfluenced, as suggested by LEWIS[6].

Pilot experiments, recently performed in our laboratory, indicated that the feeble rat paw oedema induced by synthetic bradykinine was not inhibited by pretreatment with phenylbutazone.

The observations presented offer the possibility of developing a method that would be suitable for the study of drug influences on the release of plasma kinin-like materials. For this purpose, however, some intercurrent variables must be eliminated. One of these is the tendency of the oedema fluid's biological activity to decline and present experiments indicate that we are well on the way to solving this question.

*Zusammenfassung.* Ein biologisch aktives Prinzip in der Ödemflüssigkeit aus Rattenpfoten nach Serotoninbehandlung ist wahrscheinlich nicht Serotonin oder Acetylcholin, sondern zum geringeren Teil eine histaminähnliche, zum grösseren Teil jedoch eine Substanz, die ähnliche Eigenschaften wie Bradykinin zeigt.

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*Department of Pharmacological Research, N.V. Organon, Oss (The Netherlands), September 23, 1964.*

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