with intrasplenic pellets of the halogenated progesterone derivative. Thus the vagina, which opens under the action of œstrogen, was found closed in eighteen out of nineteen animals; the symphysis pubis opened in five out of nine animals in group *A*, and in eight out of nine animals of group *B*.

There is one objection against the conclusion that the fluoro derivative resists inactivation in the liver. In six out of nineteen animals of groups *A* and *B* there were considerable adhesions between the spleen and the abdominal wall. The question of adhesions in similar experiments has been already discussed on former occasions[3]. It is not likely that results have been stultified by the adhesions.

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Influence of Hexanitrodiphenylamine on the Incidence of Neoplasms in the Mammary Tissue of Rats

The industrial use of hexanitrodiphenylamine (dipicrylamine, DPA) and the consequent exposure of human beings to it led us to study the chronic toxicity of this compound in experimental animals. In the first part of a feeding test with rats, the substance appeared to have a low toxicity; but towards the end of the life-span of animals which were fed with large amounts of hexanitrodiphenylamine, we were surprised to see that all the females had big and often multiple mammary tumours. These were fibroadenomata, adenofibromata or fibromata of the same type as occur spontaneously in our rats.

In a following experiment, hexanitrodiphenylamine was given to rats, directly after weaning, in a dose of 500 p.p.m., mixed into the dry food. The diet consisted of two-thirds whole wheat meal, one-third of whole dried milk powder and 1 per cent of a salt mixture. Food and water were given *ad libitum*; green fodder twice a week was included. The rats were derived originally from the Wistar strain, but brother–sister mating has not been continued during the past ten years.

Since hexanitrodiphenylamine can easily be hydrolysed *in vitro* to picric acid and picramide (2,4,6 trinitroaniline), these substances were included in the test, using the same dosage of 500 p.p.m. At the end of about two and a half years, the experiment had to be terminated. In the male animals, at autopsy, a variety of tumours were observed. Since neither incidence nor type of these was different from the usual, these animals will not be considered further. In the females, the occurrence of tumours of the mammary glands in the different groups is summarized in Table 1.

Table 1. Incidence of Mammary Tumours in Female Rats treated with Hexanitrodiphenylamine.

Dosage, 500 p.p.m. mixed in dry food. The numbers in brackets indicate total incidence as found in our untreated rats, including that from other experiments during the same period; see text

Group	No. of rats	No. alive after 2 years	No. with mammary tumours	Average age of appearance of tumours
Controls	19 (83)	12 (63)	8 (18)	25 months (26)
Hexanitrodiphenylamine	10	1	10	19 months
Picric acid	10	7	4	22 months
Picramide	8	7	4	29 months

From these observations it appears that in all groups the incidence of mammary tumours is high. Since in the control rats the frequency is much higher than we are accustomed to see, we have included (in brackets) the corresponding data from other untreated control rats, collected from a series of different toxicity experiments in this laboratory with the same kind of rats and diet and from the same period.

Again, in this experiment, every female rat treated with hexanitrodiphenylamine developed mammary tumours, which were found at autopsy, on the average at the age of nineteen months, which is equal to the life-span. In the control rats the age of the appearance of tumours is twenty-five to twenty-six months, and the average life-span more than twenty-five months (nearly half the animals were still alive at the end of the experiment). Without exception all these tumours, including those of the untreated animals, were fibroadenomata, adenofibromata or fibromata. All transplantation experiments with these tumours gave negative results. Another interesting feature is that the rats treated with hexanitrodiphenylamine nearly all had multiple tumours, with an average of three per animal. In control rats the development of more than one mammary tumour in the same animal is seldom observed. Hence this substance seems to increase the rate of formation of this type of tumour in our rats to the level of 100 per cent. Since the spontaneous occurrence is already rather high, it is doubtful whether hexanitrodiphenylamine should be considered on this evidence to be a true carcinogen. In another feeding test, but with mice (F_1 generation of *DBAf* × 0 20) and the same dosage (500 p.p.m.) no effect has been observed within a period of 21 months. This experiment is still going on.

In the case of picric acid and picramide, the incidence of mammary tumours did not differ from the control animals. It seems, therefore, that these products are not responsible for the effect observed with hexanitrodiphenylamine. Rats treated with high dosages of hexanitrodiphenylamine soon develop a purple-brown discoloration of nearly all tissues, and especially of the testis. This effect was not observed with either picric acid or picramide. The composition of the pigment responsible for the discoloration is now being examined.

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Copulation in Calanoid Copepods

In the course of picking out copepods for experiments on their feeding, respiration and other activities, I have made a number of incidental observations on the manner of pairing in four species of calanoids (*Centropages hamatus*, *Temora longicornis*, *Eurytemora velox* and *Acartia clausi*), which so far as I know has previously been described in only one calanoid genus, *Diaptomus*[1].

In all these species the process of pairing and the transfer of the spermatophore are substantially the same. Whether the male actively pursued the female could not be determined; the first event in the pairing which was observed was the seizing of the female by the male. This was done by the same means and in nearly the same position on every occasion when it was observed. The male held the female around the terminal segment of the urosome or the caudal rami by means of the right geniculate antennule, the distal part of which was so far reflected that its terminal segments crossed the middle region of the antennule. A pair of copepods might remain for several minutes in this position, and two specimens of *Eurytemora* remained paired in this position for several days in a watch-glass. As the pair swam about, the female made vigorous efforts to shake off the grip of the male and often succeeded. For his part, the male attempted to exchange his first grip for a second in which he gripped the female either immediately in front of, or immediately behind, the genital somite by means of the fifth leg or legs (figures of the fifth legs of these species are given by Sars[2] and Gurney[3]). In most pairs the two animals were lying head to tail with their ventral sides opposed, but in one pair of *Centropages* both had their dorsal sides uppermost and their urosomes so flexed that the genital somites were opposed. *Centropages* habitually holds its urosome dorsally flexed, so that this is a more natural position for copulation in this genus than might at first appear. Since the animals often 'danced' violently in this phase of pairing, it was sometimes difficult to make out the exact position in which they were lying.

It was very easy to determine whether or not a spermatophore had been transferred after the pair had separated, since the spermatophore attached to the genital somite is easily seen; but the actual transfer was never observed. Sars[2] says of *Euchaeta*, and Wolf[1] of *Diaptomus*, that the spermatophore is placed on the genital somite of the female by the left fifth foot of the male. I found some preserved males of *Euchaeta norvegica* in which the minute forceps at the tip of this leg was fixed holding the neck of a spermatophore, but I was unable to determine whether a forceps of this kind was used in the species I observed.

Females examined after copulation had only a single spermatophore attached to the genital somite, and usually the pair separated after this second stage of copulation. In a few cases, however, after transfer of the spermatophore the male reverted to his first grip, holding the urosome of the female with his antennule. Although it was never actually seen, it is possible that a male might clasp the female again with the fifth leg and transfer a second spermatophore—three or four are occasionally found on one female.

The species in which pairing has been described belong to widely separated families of the Calanoida[2,3], but the course of events in pairing is identical in all of them. It seems reasonable to infer that pairing follows very much the same course in all the calanoids which have geniculate antennules and clasping fifth legs, that is, in twelve of the twenty-six families into which Gurney[3] divided the Calanoida. In most of the remaining families the fifth legs clearly form a clasper which, like that of *Euchaeta*, is used in the way suggested above, but the antennule is not geniculate and we have no information about the first phase of copulation.

The observations described were made in the Zoology Department, University of Southampton, and in the Marine Station, Millport.

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Growth of Fowl Plague Virus in Macrophages and Giant Cells

The fowl plague virus strain 'Rostock' has been grown in monolayer tissue cultures of macrophages. The virus grows rapidly in these cells, producing characteristic cytopathogenic effects.

The macrophage tissue cultures were prepared from heparinized whole chicken blood which was centrifuged to isolate the white blood cells. After 18–24 hr. incubation the cultures are thoroughly washed with Earle's saline, which removes any remaining red blood cells since only the leucocytes firmly adhere to the glass. The nutrient medium throughout this work consisted of 20 per cent chicken serum, 5 per cent embryo extract, and 75 per cent Earle's saline. The Earle's saline was buffered to maintain a *p*H in the carbon dioxide incubator between 6·5 and 7·0.

The growth of fowl plague virus on 4–5-day old macrophage monolayers has been studied by measuring the release of new infectious particles into the medium, the infectious units being assayed by the Dulbecco plaque technique on chick embryo fibroblasts[1]. After a latent period varying between 1 and 2 hr., there is an exponential release of virus which lasts between 3 and 4 hr. The cells are drastically altered during this time. Already after 1 hr. they cannot be stained by the vital dye, neutral red. Between 6 and 8 hr. after the start of infection, practically all the cells have rounded up and are destroyed.