

## THE INHALATION OF HEXOESTROL

BY

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The word gynaecomastia was first used by Galen to describe an enlargement of the male breast (Karsner, 1946). Such a condition may occur after the administration of natural oestrogens (Dunn, 1935). It occurred, with increased pigmentation of the areola and nipple, in a young man treated for acne with injections of oestradiol benzoate (Foss, 1940). Dunn (1940, 1941) reported gynaecomastia after 5 mg. of stilboestrol daily by mouth for about 2–3 months in sexual criminals. Reports have since been made of similar effects after synthetic oestrogen therapy for prostatic carcinoma (Moore, Wattenburg, and Rose, 1945) and for acne (Simon, 1945).

Scarff and Smith (1942) described two cases occurring in a factory where the highly potent synthetic oestrogen stilboestrol was manufactured in quantity, even when stringent measures of protection were adopted. Occupational gynaecomastia then became a recognized entity, and Fitzsimmons (1944) has described the clinical findings in 20 cases, together with the routes of absorption and the preventive measures taken.

In the early months of 1942 we were interested in this industrial hazard, which naturally was causing a little concern among the workers, as in addition to the gynaecomastia there was the real risk of impotence and testicular atrophy, if only of a temporary nature (Dunn, 1940, 1941), and possibly of malignant change occurring in breasts showing "a dangerous degree of epithelial proliferation" (Scarff and Smith, 1942).

After ingestion of natural oestrogens, much of the activity of a given dose is lost by detoxication in the liver, and it was shown by Biskind and Mark (1939) that a pellet of oestrone implanted in the spleen, and thus absorbed via the liver, was considerably less efficient in producing positive vaginal smears in castrated female rats than a pellet of the same size and shape implanted subcutaneously. Stilboestrol and other synthetic oestrogens probably behave somewhat differently and are effective by the mouth.

Workers in factories where synthetic oestrogens are made must therefore be protected against the risk of swallowing small quantities, or of absorbing them by the skin. They are also liable to be exposed to the inhalation of fine dusts contaminated with oestrogens. The work described below was undertaken in

order to assess the effects of such exposure and to compare the effects with those of similar doses by the mouth.

### METHODS

Experiments have been made with male human volunteers and with guinea-pigs. With men no special methods were used, but with guinea-pigs the effect was measured.

Male guinea-pigs show well-marked growth of the nipples with development of mammary tissue after a total dose of 0.2 mg. of hexoestrol has been injected subcutaneously every other day for 20 days (Dodds, 1939). In the experiments described here, light-coloured or white guinea-pigs weighing 300–700 gm. were clipped around the nipples, and were then anaesthetized with ether and photographed under constant conditions. The right nipple was held lightly resting on a fixed and rigid millimetre scale, with white divisions on a black background. The source of light was constant, and the same aperture and the same exposure were used each time. These photographs were all enlarged 7 times, and the length of the nipple and the diameter through the centre of its length were measured by comparison with the photographed scale. The volume of the nipple was then calculated on the assumption that it was a cylinder; the tapering at the tip was roughly balanced by the broadness of the base. The volume of the nipple was measured in this way before treatment and 14 days later, and the percentage increase in volume was calculated.

Hexoestrol was administered in solution in arachis oil, either by stomach tube or by inhalation of the spray of a Collison (1935) inhaler. The spray was directed down a wide glass tube with a large hole in the side of it, partly closed by a rubber diaphragm, through which the guinea-pig's nose was poked. The dose of hexoestrol inhaled by the guinea-pigs was roughly estimated in the following way. The output of the spray was 9.4 litres of air and 120 mg. of oil per minute, so that the concentration of the oil in the cloud breathed by the guinea-pig was about 12.8 mg./litre. It was assumed that the guinea-pigs, which weighed about 500 g., breathed 250 c.c. of air or 3 mg. of oil per minute (Gaddum, 1944).

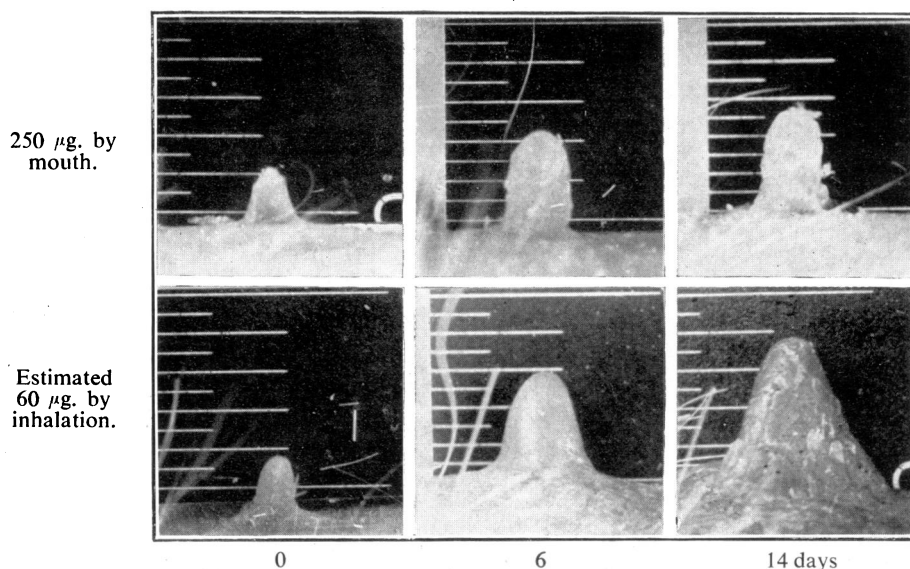


FIG. 1.—Nipples of guinea-pigs before and after a single dose of hexoestrol in arachis oil. Scale, mm. and 0.5 mm.

The dose of hexoestrol was then calculated from the known concentration of the drug in the oil and the duration of the exposure. The assumptions involved make these estimates of the dose unreliable. A small amount of hexoestrol was deposited on the walls of the apparatus and the actual dose inhaled was probably less than the estimate, but it seems likely that it was more than 50 per cent of the estimate.

## RESULTS

*Guinea-pigs.*—In two experiments the effect by the mouth was compared directly with the effect by inhalation. Guinea-pigs which inhaled hexoestrol in the first experiment were not photographed individually before the treatment,

TABLE  
GUINEA-PIGS. EFFECTS OF HEXOESTROL ON NIPPLE VOLUME

Weight gm.	Approx. dose $\mu$ g.	Time of exposure min.	Normal nipples			After 14 days			% inc.	Remarks
			Length mm.	Width mm.	Volume cu.mm.	Length mm.	Width mm.	Volume cu.mm.		
EXPERIMENT 1.										
<i>By mouth</i> using 0.1% and 0.01% solution in Arachis Oil.										
570	50	—	1.7	1.2	1.92	2.9	1.6	5.8	202	
710	100	—	1.8	1.1	1.69	3.4	1.6	6.8	243	
520	250	—	1.5	1.2	1.70	3.3	1.8	8.4	390	
600	500	—	1.7	1.1	1.60	4.0	1.7	9.0	460	
550	750	—	2.2	1.2	2.49	4.8	2.0	15.0	500	
660	1000	—	1.7	1.0	1.34	4.0	1.9	11.2	735	
<i>By inhalation</i> using 1% solution in Arachis Oil.										
355	30	1				3.1	2.4	14	610	
305						—	—	—	—	Died 6 days
305	60	2			1.92	4.3	2.5	21	1000	
400					(Mean of 26)	3.9	2.8	24	1150	
390	120	4				3.3	2.0	10.4	440	
330						4.0	2.2	15.2	690	
EXPERIMENT 2.										
<i>By mouth</i> using a 0.001% solution in Arachis Oil.										
520	2.5	—	1.8	1.0	1.34	1.8	1.0	1.34	0	
650		—	2.6	1.3	3.4	2.5	1.2	2.8	—18	
550	5	—	2.4	1.2	2.7	2.4	1.1	2.25	—17	
500		—	1.7	1.0	1.33	—	—	—	—	Died 5 days
620	10	—	2.2	1.1	2.1	2.2	1.2	2.5	19	
530		—	2.0	1.1	1.9	2.1	1.1	2.0	5.2	
600	25	—	2.0	1.1	1.9	2.3	1.2	2.6	37	
600		—	2.0	1.1	1.9	2.1	1.2	2.38	25	
700	40	—	2.5	1.5	4.4	—	—	—	—	Died 11 days
520		—	1.9	0.9	1.2	1.9	1.1	1.8	50	
<i>By inhalation</i> using a 0.1% solution in Arachis Oil.										
520	0	0	1.7	1.2	1.9	1.7	1.1	1.6	—16	
530			2.2	1.5	3.9	—	—	—	—	Died 16 days
600	3	1	2.0	1.4	3.1	2.2	1.4	3.38	11	
480			2.2	1.4	3.4	2.5	1.4	3.85	13	
590	6	2	1.8	1.0	1.4	2.8	1.4	4.15	195	
540			1.9	1.3	2.5	2.1	1.6	4.2	68	
500	12	4	2.2	1.2	2.5	4.0	1.8	10.1	300	
420			1.4	0.9	0.88	2.5	1.5	4.4	250	Died 31 days
—	24	8	1.2	0.9	0.75	—	—	—	—	Died 16 hr.
650			1.5	1.3	1.98	2.4	1.6	4.8	143	

and the percentage increase in volume was calculated by comparing the volumes after treatment with the average volume of 26 normal nipples, belonging to guinea-pigs of about the same weight. With this exception the volume of each nipple was estimated before and after treatment. The results of these experiments are shown in the Table and Figures.

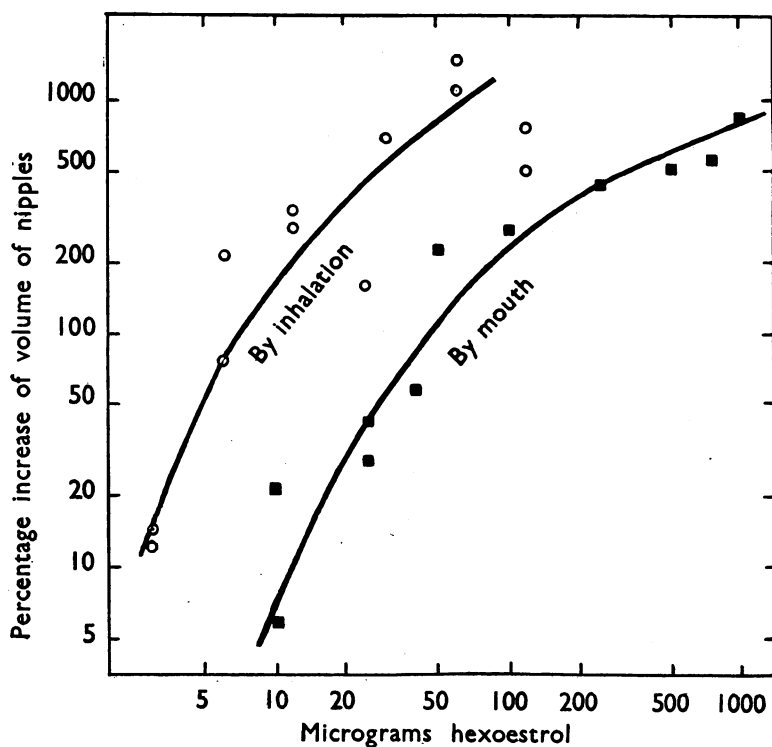


FIG. 2.—Effects of hexoestrol given to guinea-pigs by inhalation (circles) and by the mouth (squares). Logarithmic scales. Horizontally, the estimated dose in micrograms per animal. Vertically, the percentage increase of the volume of the nipples after 14 days.

The deaths recorded in the table were probably not due to hexoestrol. Deaths occurred, among normal guinea-pigs in the stock, rather frequently during this period. The figures are erratic owing to the normal variation between guinea-pigs and the smallness of the numbers used, but they suffice to show approximately the threshold doses for a single dose by the two methods of administration. Hexoestrol in oil was 5–10 times as effective by inhalation as it was by the mouth.

*Man.*—A series of 7 male observers were given single doses of 15–50 mg. of stilboestrol by mouth without any effect on the nipples or breasts at all. Some of these men, consisting of professors, doctors, and laboratory assistants, noticed

loss of appetite, slight headache, and malaise. One doctor took 55 mg. in 4 days without any effect at all on the nipples. A laboratory assistant swallowed 5 mg. a day for 17 days. He noticed some indigestion and an empty feeling in the epigastrium, and at the end of this time he said that he was conscious of his nipples being rather sensitive and he felt the pressure of his pyjamas on them. On examination the areola were bright pink and there was slight enlargement of the nipples, but there was no thickening of the breast tissue beneath. He then stopped taking tablets, as he felt a little anxious.

#### DISCUSSION

The experiments on guinea-pigs show that hexoestrol in arachis oil is very effective by inhalation. The effect shown in Fig. 1 was due to exposure for only 2 min. altogether to a concentration of hexoestrol estimated as 128 mg. per cubic metre. The curves shown in Fig. 2 suggest that the dose for a given effect by inhalation is only about 1/5th of the dose for the same effect by oral administration. If the conclusions given above about the error of the estimate of the amount inhaled are true, then the dose by inhalation lies between 1/5th and 1/10th of the dose by oral administration. It is possible that the large effects observed after inhalation were due to the formation of a depot of arachis oil in the lungs from which the hexoestrol was slowly absorbed.

The nipples of man appear to be less sensitive than those of the guinea-pig when hexoestrol is taken by the mouth and when doses are calculated in mg. per kg. Thus a dose of 0.25 mg. or 0.5 mg. per kg., given by the mouth to a male guinea-pig, increased the volume of the nipple five times. The length of the nipple was more than doubled and the general effect was definite, but not maximal. On the other hand, a dose of 50 mg., or nearly 1 mg. per kg., had no observed action on man. The drug is probably more effective when given in repeated small doses, but it is apparent from Dunn's experiment and from those recorded here that 5 mg. per day is only effective if continued for several weeks.

There is no quantitative information available about the effects of inhalation by man, and calculations about this must be based on indirect evidence. A dose of 5 mg. of hexoestrol per day by the mouth is likely to lead to troublesome effects if continued. Judging by the experiments with guinea-pigs, this would be equivalent to between 0.5 and 1 mg. per day by inhalation. If a man inhales 15 litres per minute, he will inhale 7.2 cu.m. in a working day of 8 hours. It would therefore be expected that a concentration of 0.5–1 mg. in 7.2 cu.m., or 0.07–0.14 mg. per cu.m., would be harmful after several weeks if exposure were continuous for 8 hours a day. Fitzsimmons (1944) observed a concentration of 0.2 mg. of stilboestrol in 300 cu.ft., or 0.023 mg. per cu.m., on one occasion in the Glaxo laboratory. Our calculations suggest that such concentrations of hexoestrol would not be immediately harmful, but are near the dangerous limit if maintained for several weeks.

Fitzsimmons found that the vaginal smears of ovariectomized mice became positive when they were kept for 3 days in various parts of the factory where stilboestrol was being prepared, even when careful precautions were taken to prevent the dispersal of the oestrogen in the air. An alternative biological test is now available. If male guinea-pigs were kept in factories where synthetic oestrogens are made, their nipples could be inspected, and if necessary measured, at regular intervals and so used as an index of danger. This would be simpler than the vaginal smear technique, though presumably less sensitive. It is probable, however, that the smear test is too sensitive and becomes positive when there is no danger. There is reason to believe that the guinea-pig's nipple will provide an indicator of oestrogens in the air which is sensitive enough for practical purposes. The guinea-pig is more sensitive than man to hexoestrol as judged by the oral dose per kg. of body weight; he inhales more air, and thus more oestrogen, per kg. of body weight and is thus likely to be relatively even more sensitive to clouds than to oral administration. If a man and a guinea-pig were exposed daily to clouds of oestrogen together, the guinea-pig should therefore be affected first and give adequate warning to the man.

The results also suggest a method for the therapeutic administration of oestrogens. They have been given by ingestion, injection, inunction, and implantation. They might also be given by inhalation, but it would probably be difficult to control the dosage and there would be no obvious compensating advantages in this method of administration.

#### SUMMARY

1. A solution of hexoestrol in arachis oil was administered to male guinea-pigs and the effect on the nipples was photographed and measured.
2. A given dose had more effect when given by inhalation than when given by the mouth.
3. It is suggested that male guinea-pigs should be kept in places where synthetic oestrogens are made, to give warning of any failure of the measures taken to protect the workers from the effects of the oestrogens.

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