history of premature infants with large Tween 80 supplements in the diet.

Summary. Two non-ionic detergents, Tween 80 and Triton X-100, have been compared with respect to their toxicity to chick embryos. Tween 80 was found to be 20 times less toxic than Triton X-100. The possibility is considered that with the increasing age of the embryos there is a decrease in the toxicity of both detergents.

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1. Morton, H. J., Morgan, J. F., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 22.

2. Krantz, J. C., Jr., Culver, P. J., Carr, C. J., and Jones, C. M., *Bull. School of Med.,* Univ. Maryland, 1951, v36, 48.

3. Holtfreter, J., *J. Exp. Zool.,* 1943, v93, 251.

4. Dubos, R. J., and Middlebrook, G., *J. Exp. Med.,* 1948, v88, 81.

5. Glassman, H. N., *Science,* 1950, v111, 688.

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Effect of Repeated Doses of External and Internal Irradiation on Structure of the Spleen.* (20127)

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It has been pointed out(1) that the spleen is particularly adapted to a study of susceptibility of various cells to irradiation. This statement can be further extended by indicating that pathological findings in the irradiated spleen are suitable to illustrate the fate not only of cellular elements, but also of the structure of a parenchymatous organ, containing numerous free cells of various kinds in a framework of connective tissue, as a whole. For this purpose, a larger amplitude of changes could be produced by using for internal irradiation colloidal radioactive gold Au^{198}† which is short living (half life of 2.7 days), beta emitter and which can be deposited, owing to its colloidal structure(2) into the spleen, in graded amounts. This method, alone or in combination with external irradiation, enabled us to obtain in experimental animals a larger variety of changes in the spleen structure than those elicited by external irradiation alone(3) as is shown by the data presented below.

Material and methods. (a) *Animals.* Mice of C3H strain,‡ of 24 to 27 g weight, mostly males, were used throughout in these experiments. For internal irradiation they received injections of a radioisotope intraperitoneally, intrapleurally (the technic of this injection was described elsewhere(4)), intravenously or subcutaneously. For external irradiation, groups of not more than 12 mice were immobilized in horizontal position between 2 cardboard sheets inside a small box adjustable to irradiating appliance. (b) *Internal irradiation.* Colloidal Au^{198}† was injected in a volume of 0.5 cc containing 0.01 to 0.03 millicurie (small doses) or 2 millicuries (large dose). (c) *External irradiation.* Doses of 100 to 1200 r of filtered rays (Filter: 1.2 mm Sn; 0.25 mm Cu; 1.0 mm Al; TSD 40 cm; HVL 3.27 mm Cu; 250 PKV) were used. (c) *Autopsies.* Preliminary research provided information about time interval between exposure and effect, for each type and amount of irradiation, thus indicating the suitable interval between exposure and autopsy in the main experiments.

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† Radioactive Au^{198} was obtained through Dr. D. L. Tabern, Abbott Research Laboratories, North Chicago, Ill.

‡ The mice were obtained from Roscoe Jackson Memorial Laboratories, Bar Harbor, Me.

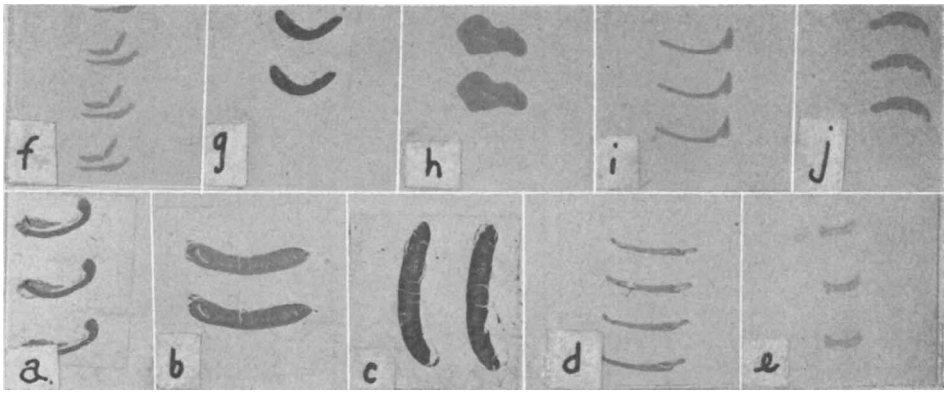

FIG. 1. Changes in size of the spleen of the mouse after irradiation (natural size of stained sections). (a) normal spleen; (b) five days after inj. of 0.01 mc of radiogold colloid H + E; (c) same, Mallory; (d) three days after inj. of 2 mc of radiogold colloid; (e) nine days after inj. of 0.01 mc and 3 days after subsequent inj. of 2 mc of radiogold, H + E; (f) three days after whole body exposure to 1200 r, H + E; (g) Nine days after inj. of 0.01 mc of radiogold and 3 days after exposure to 1200 r; (h) three days after 2 exposures to 400 r at interval of 7 days, H + E; (i) three days after 8 exposures to 100 r at intervals of 3 or 4 days; (j) three days after 4 exposures to 200 r at intervals of 1 day.

The spleens were stained with hematoxylin-eosin or by Mallory technic.

Results. I. *Effect of a small dose, single or repeated.* A. *Internal irradiation.* Four days after intrapleural or intraperitoneal injection of 0.01 to 0.05 mc of colloidal Au^{198}, the spleen was found 5 to 8 times larger (Fig. 1, b and c) as compared with an average normal spleen (Fig. 1a). Microscopic examination revealed high congestion of the organ with marked distention of connective tissue framework and of the capsule. However, lymphocytopoietic centers were conspicuous among the pulpal elements rarified by congestion of the organ. The congestion disappeared as a rule within 7 to 14 days leaving the spleen slightly enlarged and impregnated with deposit of iron pigment. Repetition of same doses of radiogold at any intervals would not increase the size of the spleen and its congestion beyond the effect of a single dose, but it would decrease the number of lymphocytic elements. B. *External irradiation.* Doses of 50 to 250 r did not induce any noticeable change in the size of the spleen. Slight increase (less than twice the size of an average spleen) was noted within 4 days after irradiation with 150 to 400 r. After repeated doses of 200 or 400 r sufficiently spaced the spleen showed enlargement by congestion (Fig. 1, h and j), while after frequent application of even smaller doses, it was shrunken (Fig. 1, i). These experiments with various small doses of x-rays variously spaced will be reported separately(5).

II. *Effect of a high irradiation dose.* A. *Internal irradiation.* A dose of 2 mc of radiogold colloid was lethal for all mice within 5 days. In sacrificed moribund animals the spleen size was ½ or ⅓ of an average normal spleen, the shrinkage being most marked in both poles (Fig. 1, d). The tissue sections presented microscopical evidence of shrinkage—contraction of connective tissue framework and separation of the capsule from the organ surface—and of congestion. Both changes were diffusely marked in the red pulp and on the periphery of the spleen, while the white pulp containing lymphocytes with pyknotic nuclei was apparently "condensed" by the shrinkage of the whole organ. B. *External irradiation.* Exposure to 1200 r was followed by the death of all mice on the 4th day with few exceptions surviving until the 5th day. At autopsy, the spleen was about 1/5 or 1/6 of the average size of the normal spleen. The shrinkage was manifested by constriction of the body of the spleen (Fig. 1, f) and microscopically by marked outlines of connective tissue bundles and by separation

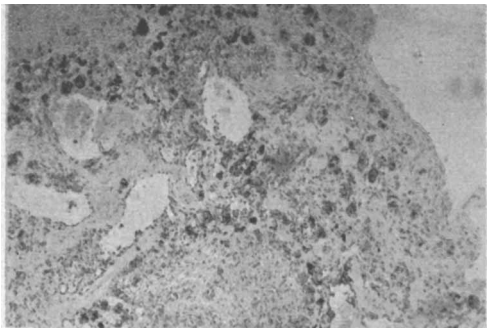

FIG. 2. Spleen of the mouse, 9 days after inj. of 0.01 mc and 3 days after subsequent inj. of 2 mc of colloidal radiogold. H + E × 50.

of the capsule from organ surface. The congestion was not marked and lymphocytic elements appeared considerably reduced in number throughout the area of the section.

III. *Small irradiation dose followed by a large dose.* (A) *Small dose internal irradiation followed by a large dose.* This method of irradiation is a combination of IA and IIA, *i.e.,* 1) the injection of 0.01 mc of radiogold colloid which was shown to induce spleen enlargement by congestion followed after 5 or 6 days by 2) the injection of 2 mc, which alone caused shrinkage and partial atrophy of the spleen. Autopsies performed on the 4th day after the 2nd injection revealed the striking effect of this combined treatment; within 96 hours the size of the spleen was reduced more than 10 times (from that shown in Fig. 1, b and c, to that of Fig. 1, e). Moreover, already the gross examination of stained spleen sections (same figure) revealed the disappearance of the pulp within the shrunken framework of the organ. This was confirmed by microscopic examination (Fig. 2), which showed nearly complete disappearance of all cellular elements with the exception of fibrous tissue and of numerous macrophages loaded with gold particles and iron pigment. Briefly, this phenomenon can be described as complete spleen atrophy induced in a mouse within 8 or 9 days by successive injection of a small and a large dose of colloidal radiogold. B. *Small dose of internal irradiation, followed by a large dose of external irradiation.* In this experiment mice pretreated with a small dose (0.01 mc) of radiogold, as above, received on the 5th or 6th day a large dose of external irradiation (1200 r) instead of radiogold. The autopsy on the 4th day after the 2nd treatment indicated that the spleen, which was highly congested after the injection of small Au^{198} dose, returned to approximately its normal size (Fig. 1, g) and did not shrink beyond it, as it was recorded above after reinjection of radiogold (2 mc) in pretreated mice. Microscopically the diffuse and extensive atrophy of the pulp was less advanced than in the above experiment (Fig. 2) and there was a marked thickening of fibrous tissue bundles in the organ framework and in its capsule. Thus, the pretreatment of a mouse with a small dose of radiogold did increase the severity of pulp destruction and framework shrinkage by external irradiation, but not so dramatically as in the above experiment.

Several series of mice were exposed to a small amount of external irradiation (100 and 250 r) and after 5 days injected with 2 mc radiogold or exposed to 1200 r. In each series the effect of combined treatment on the spleen was not significantly different from the effect of the large dose of irradiation alone. Therefore, a small dose of external irradiation had no or only a slight preparative effect on the results of subsequent treatment with massive irradiation.

Discussion. Experimental work in mice on histopathology of spleen irradiation has been reviewed by Dunlop(6) and by Murray(1). It is the consensus of opinion that after external irradiation the spleen undergoes brief early swelling and then shrinks rapidly. The initial congestion was rapidly transient; for instance, after irradiation of the mouse with 80 r some changes were found in the spleen after 4 hours, but none after 24 hours(1). The effect of 100 to 300 r was only slightly more marked. After exposure to 600 r or over, the organ was found shrunken(1) and fibrotic(6), with enormous number of macrophages.

Internal irradiation with a soluble radioisotope—Strontium^{89}—did not produce even in high doses (0.036 mc per g weight) any significant changes in the spleen(1). However, relatively small doses (0.23 to 0.26 mc) of a particulate radioactive material—chromic

phosphate with half life of 14.3 days—"reduced within 15 to 23 days the spleen almost to a connective tissue framework and macrophages"(7). This difference in the effect of soluble and of particulate radioisotopes depends obviously on the retention of the latter in the spleen. The particulate radioisotope used in our experiments—colloidal Au^{198} with half life of 2.7 days—was found to be suitable to produce graded changes in the structure of the spleen. This advantage can be accounted by shorter half life and smaller particles of colloidal radiogold.

The high congestion induced in the spleen by minimal doses (0.01 to 0.05 mc) of colloidal Au^{198} was, obviously analogous to the initial effect of external irradiation, but more marked and more lasting, owing to protracted action of deposited radioactive colloid on tissues.

The rapid shrinkage of the spleen after injection of high doses of the same isotope was similar to the effect of high doses of external irradiation. As it would be expected, numerous radioactive particles diffusely spread throughout the tissue produced more severe and more extensive changes in the spleen structure than higher, but short acting doses of external irradiation.

It was found unexpectedly that successive injections at a very short time interval, of a minimal dose (0.01 mc) and a 200 times larger dose (2.0 mc) of colloidal radiogold induced rapidly a complete atrophy of the spleen, leaving only the connective tissue framework and the pigment and gold loaded macrophages. Obviously, a minimal dose of radiogold incapable by itself to induce significant regressive changes in the spleen had some preparatory action on the organ by rendering its tissues more susceptible to the subsequent blow inflicted by a high dose of radiogold. There is some evidence in the work of several authors (6) that the radiosensitivity of the spleen increases with increase of the blood supply. It can be presumed therefore that the preparative effect of the small dose of radiogold is due to its remarkable lasting congestive effect on the spleen. This interpretation will agree with our finding that small doses (50 to 200 r) of external irradiation which induce only slight and transient congestion of the spleen or none did not exert "preparative" effect on the spleen treated subsequently with high doses of radiogold or of x-rays. It may be concluded that high congestion of the spleen induced by a small dose of colloidal radiogold constituted "the preparative factor" responsible for rapidly progressing severe atrophy of the spleen after injection of a high dose of radiogold into the same mouse.

It should be emphasized that both the high congestion and the advanced atrophy of the spleen were induced regularly only in one strain of mice—C3H and by intrapleural or intraperitoneal injection. It may be presumed that these findings can be reproduced in other animal species and by other routes (intravenously) by varying the dosage, the interval between injections and perhaps some other conditions of the experiment.

Summary and conclusions. 1. Minimal doses of internal irradiation (0.01 to 0.05 mc of colloidal radiogold) induced in C3H mice within 5 or 6 days a very marked congestive swelling of the spleen which lasted for about 14 days. No significant effect on size of the spleen could be obtained within a wide range (50 to 200 r) of small doses of external irradiation. 2. Large doses of radiogold (2 mc) as well as of external irradiation (1200 r) caused shrinkage and partial atrophy of the spleen within 3 days, the effect of the former being more severe. 3. Large dose of colloidal radiogold (2 mc) injected 5 or 6 days after pretreatment with a minimal dose (0.01 mc) of the same radioisotope induced within 3 days more marked shrinkage and more complete atrophy of the spleen than the same dose of radiogold without pretreatment. Substitution of radiogold (either of a preparative dose or of a large dose) by analogous doses of external irradiation did not reproduce the same drastic changes of the spleen. 4. These findings were interpreted in the light of the existing evidence that the radiosensitivity of the spleen increases with the increase of the blood supply. It was presumed that high congestion of the spleen induced by a small dose of colloidal radiogold constituted "the preparative factor" responsible for rapidly progressive severe atrophy of the spleen after subsequent

injection of a high dose of radiogold into the same mouse.

1. Murray, R. C. The Spleen, in "Histopathology of Irradiation from external and internal Sources". Edited by W. Bloom, McGraw-Hill, 1948.

2. Hahn, P. F., and Sheppard, C. W., *So. Med. J.*, 1946, v39, 558.

3. Clarkson, J. R., Mayneard, W. V., and Parsons, L. D., *J. Path. Bact.*, 1938, v46, 221.

4. Goldie H., Jeffries, B. R., Maxwell, M. C., and Hahn, P. F., *Cancer Research*, 1952, v12, 422.

5. Tarleton, G. J., Goldie, H., Jones, A., and Moore, G. A., in press.

6. Dunlop, C. E., *Arch. Path.*, 1942, v34, 582.

7. Jones, H. B., Wrobel, C. J., and Lyons, W. R., *J. Clin. Invest.*, 1944, v23, 783.

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In vitro and *In vivo* Activity of Candicidin on Pathogenic Fungi. (20128)

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Lechevalier, Acker, Corke, Haenseler, and Waksman(1) have established that candicidin is an antifungal agent with activity against certain filamentous and yeast-like fungi. The purpose of the present communication is to define the antifungal spectrum of the water-soluble fraction of candicidin against pathogenic fungi and to estimate its protective effect in mice with systemic mycoses.

Materials and methods. The antibiotic was dissolved in various concentrations in an agar medium containing 2% glucose and 1% neopeptone adjusted to pH 7. A 1:1000 volumetric suspension of the various fungi were streaked over the agar surface. The plates were incubated at 37°C and at room temperature. Estimation of growth inhibition was made within 2 to 3 days after growth was evident in the control plates. This was 2 to 3 days after incubation for rapidly growing yeast-like fungi, such as *Cryptococcus neoformans* and *Candida albicans*, and 7 to 8 days for slower-growing fungi such as *Histoplasma capsulatum* and *Coccidioides immitis*. To determine fungicidal activity a suspension of *C. albicans* in brain heart infusion broth containing approximately one million cells/ml was prepared. The antibiotic was then added in concentrations of 10 and 50 μg/ml. At 2, 4, 6, 8, and 24 hours samples of the broth were removed and the quantity of viable cells determined by plate counts on glucose peptone medium. The heart infusion broth was maintained at 37°C during the test. The final concentration of antibiotic in the media on which the colony counts were made was not sufficient to exert a fungistatic effect.

Experimental infections. Throughout, 20 g white, male mice were used. In some cases injections of the fungi were made intraperitoneally and, in others, intravenously by way of the tail vein. The quantity of injected cells was appropriately regulated so as to avoid overwhelming infection and rapid death. The aim for the most part was to produce sublethal infections of sufficient severity to enable gross comparisons when the animals were sacrificed. The candicidin was administered intraperitoneally in all instances. Generally, it was given for 10 successive doses beginning on the day of infection. The animals were sacrificed on the 14th day.

Results. In vitro antifungal activity. Preliminary studies showed that the antibiotic activity was considerably reduced under acid conditions. At pH 5 and pH 6 approximately 100 times more antibiotic was required for inhibition than at pH 7 and pH 8, in which range the activity was maximal. Human blood slightly diminished the fungistatic power of candicidin. The antifungal activity was slightly greater at room temperature than at 37°C. Comparable results were obtained with broth and with agar streak methods. The antibiotic activity was maintained undiminished in brain heart infusion broth incubated at