be obtained by the interaction of suitable hydrogen donors and hydrogen acceptors. Aspartate is effective presumably because it is converted by aspartase into fumarate. This conclusion is supported by the fact that the presence of ammonium ions inhibits adaptation in a formate-peptone medium, but not in a glucose-*amino acid* medium where energy is secured by glucose breakdown. 2. Certain amino acids are required for the enzyme synthesis. The most effective are leucine, aspartate, histidine, and serine. Requirements for specific amino acids appear to vary with the strain of the organism.

1. Billen, D., and Lichstein, H. C., *J. Bact.*, 1951, v61, 515.
2. Pinsky, M. J., and Stokes, J. L., *J. Bact.*, 1952, v64, 151.
3. Stephenson, M., and Stickland, L. H., *Biochem. J.*, 1933, v27, 1528.
4. Quastel, J. H., Stephenson, M., and Whetham, M., *Biochem. J.*, 1925, v19, 304.
5. ———, *Biochem. J.*, 1925, v19, 660.
6. Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, 1929, v23, 115.
7. Quastel, J. H., and Woolf, B., *Biochem. J.*, 1926, v20, 545.
8. Billen, D., *J. Bact.*, 1951, v62, 793.
9. Williamson, H. B., *J. Biol. Chem.*, 1944, v156, 47.

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Toxicity of Non-Ionic Detergents for the Chick Embryo.* (20126)

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A recent investigation by Morton *et al.*(1) showed that the non-ionic detergent Tween 80 in a concentration of 0.5 mg/ml of medium inhibited the growth of fibroblasts in tissue cultures while in adult rats Tween 80 required a concentration of 5-10 g/kg of body weight(2) to be toxic. The higher toxicity of the detergent in tissue cultures raised the question as to whether rapidly growing and less differentiated cells are more sensitive to the detergent than the non-proliferating mature cells. A possibility of comparing the sensitivity to non-ionic detergents of cells in different degrees of proliferative activity and differentiation seemed to be present in the developing chick embryo. Whether non-ionic detergents had a teratogenic effect owing to their surface activity was also of interest because of the great importance of the interaction of cell surfaces during early embryonic development(3). Also, substantial amounts of non-ionic detergents such as Tween 80 are contained in various vitamin preparations which are given to premature infants as well as to mature ones. Therefore, from a clinical point of view, toxicity tests on immature tissue had some interest. Since qualitative and quantitative differences exist in the action of different non-ionic detergents upon different biological systems, such as the tubercle bacillus(4) or the red blood cell(5), it seemed desirable to compare at least two detergents of this type in their effect upon the chick embryo. We chose Tween 80† and Triton X-100† for this comparison.

Materials and methods. Tween 80 is described in the literature as polyoxethylene sorbitan mono-oleate and Triton X-100 as arylalkyl polyether of phenol. The structural formulae are given in the paper by Dubos quoted above(4). Solutions of these substances were made up in distilled water to give the desired amount of material in 0.1 ml. To reach the highest concentrations of Tween 80 the volume was increased to 0.5 ml. The control eggs were injected with 0.6% NaCl solution. All solutions were sterilized by auto-

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† We are indebted to Atlas Powder Co. for the samples of Tween 80 and to Rohm and Haas for the samples of Triton X-100.

TABLE I. Toxicity of Triton X-100 for Chick Embryos at Different Stages of Development.

Age (days incubation)	Amt of Triton inj. mg/egg	Amt of Triton inj. mg/g tissue	Total eggs inj.	% surviving
Before incubation (inj. into yolk)	.0	.0	213	67
	.1	.005	87	30
	.25	.012	194	42
7-8 days (inj. onto membrane)	.0	.0	114	82
	.12	.048	54	80
	.25	.1	59	53
	.5	.2	54	17
15-16 days (inj. onto membrane)	.0	.0	112	95
	.25	.018	20	95
	1.0	.071	54	92
	3.0	.21	23	61
	4.0	.29	45	55
	10.0	.71	24	0

claving. In most instances the solutions were injected onto the chorioallantoic membrane through a small drill hole in the eggshell which was subsequently sealed with Scotch tape. One series of experiments is listed in which the solution was injected into the yolk. The eggs were opened 3 days after injection onto the chorioallantoic membrane and 8 days after injection into the yolk. The embryos were inspected for the more obvious malformations of the head and skeleton. In view of the uncertainty of the distribution of the injected substances between yolk and embryonic tissue, our data are calculated as per whole egg or per gram of tissue of embryo plus membranes. Depending upon the calculation, the absolute values for the observed toxicities will differ. For an evaluation of the relative toxicities of the detergents which was the primary aim of the present experiments, the different calculations gave essentially the same results.

Results. Triton X-100. A dose of 0.1 mg/egg of Triton X-100 injected into the yolk previous to incubation was toxic. 67% of the controls survived, while only 30% and 42% of the experimental embryos survived the same period after injection of 0.1 and 0.25 mg of the detergent, respectively. The toxicity increased slowly with the dosage and an injection of 2.5 mg of Triton per egg reduced the number of survivors to 18%. Assuming an equal distribution of the injected compound between yolk and embryo the minimum toxic amount of 0.1 mg/egg would correspond to a concentration of about 0.005 mg/g of embryo based on the weight for the yolk plus the embryo of about 20 g on the eighth day of incubation.

In experiments in which Triton was injected onto the chorioallantoic membrane, we assume that most of the substance is taken up by the embryonic circulation. Therefore, calculations are based on the total weight of the embryo plus membranes. On the 7th-8th day of incubation 0.05 mg/g Triton had no toxic effect, 0.1 mg/g had a slight effect, and 0.2 mg/g reduced the number of survivors from 82% in the controls to 17% in the experimental embryos. On the 15th-16th day of incubation a concentration of .07 mg/g of embryo had no toxic effect, 0.2-0.3 mg/g embryo decreased the number of survivors from 95% to 61 and 55%, respectively, and 0.71 mg/g killed all 25 embryos injected (Table I). In an experiment with 24 chickens freshly hatched to one-day-old and intraperitoneal injection of 0.5 mg/g of chick killed about one-half of the chickens within 2 hours.

Tween 80. In a large series of experiments it was attempted to demonstrate toxic effects of Tween 80 on the developing embryo after injection into the yolk prior to incubation similar to the experiments with Triton X-100. However, with this technic no toxic effects could be obtained with any consistency even after injection of very large amounts (20 mg/egg). The inadequacy of this procedure could be demonstrated in experiments with embryos after 4 days of incubation. Table II shows that after injection into the yolk of 10 mg of Tween/egg the number of survivors was almost as high as in the controls. After

TABLE II. Comparison of Toxicity of Tween 80 after Injection into Yolk and onto Membrane. Age of embryos: 4 days of incubation. Amount of Tween 80: 10 mg/egg.

	Total eggs inj.	% surviving
Saline inj. onto m*	35	71
Tween 80 inj. onto m	36	33
Saline inj. into y	36	67
Tween 80 inj. into y	36	59

* m = membrane; y = yolk.

TABLE III. Toxicity of Tween 80 for the Chick Embryo at Different Stages of Development.

Age (days incubation)	Amt Tween inj. mg/egg	Amt Tween inj. mg/g tissue	Total eggs inj.	% surviving
4	.0	.0	156	79
	.25	.63	26	85
	1.0	2.5	67	19
	3.0	7.5	32	0
	10.0	25.0	85	22
7	.0	.0	374	68
	2.5	1.0	152	53
	5.0	2.0	46	54
	10.0	4.0	103	17
15–16	.0	.0	136	98
	10.0	.7	24	96
	40.0	2.9	24	92
	100.0	7.2	32	94
	200.0	14.3	49	39

injection of the same amount of Tween 80 onto the membranes, the number of surviving embryos was decreased to 33%. The negative results after injection of Tween 80 into the yolk could be due to hydrolysis of the detergent by a lipase which is known to be present in the yolk. The smallest dose of Tween 80 which caused a decrease in the number of surviving embryos when injected onto the chorioallantoic membrane at 4 days of incubation was found to be 2 mg/g of tissue. At that early stage the membranes weigh about 300-400 mg and the weight of the embryo is reported to be 50 mg.‡ At 7-8 days of incubation toxicity is still observed with 1.5-2.5 mg while at 15-16 days of incubation about 15 mg/g of embryonic tissue are required to produce even a slight toxic effect (Table III).

Discussion. Our figures give a toxicity of Triton X-100 about 20 times greater than that of Tween 80. The surface activities of the 2 compounds are: 30 dynes per cm for Triton X-100 and 41 dynes per cm for Tween 80 at a concentration of 0.1%. Triton exhibiting a lower surface tension than Tween 80, also exhibits a greater toxicity. However, it is unlikely that this difference in toxicity is simply a function of the surface activity of these substances. Sodium oleate, which has a higher surface activity than Triton, is definitely less toxic for the 7-day-old chick embryo.

In the introduction we raised the question of a dependence of the toxicity upon the age of the embryo. The fact that higher concentrations of Tween 80 and Triton X-100 are required by the 16th day of development is of interest in this connection since by this time a far-reaching maturation of many tissues has taken place. This may mean that the more mature tissue is less sensitive to the detergent. At the same time it has to be kept in mind that in the course of this maturation detoxification mechanisms may at least in part become responsible for the apparently decreased toxicity. However, our results may be suggestive enough to make it seem advisable to test other compounds of the same class of substances in order to find out whether some of them will display larger differences in their toxicity for immature and mature tissues.

In the course of experiments no occurrence of developmental abnormalities was observed and no clue has been found as to the mechanism of the toxic action of the detergents.

Concerning the question of a possible toxicity of non-ionic detergents for premature infants one has to consider that in these babies even the maximum figure for the dietary intake is at most one-tenth of the dose found lethal in chick embryos. Studies carried out by the producers of Tween 80 indicate very good tolerance of Tween 80 in men. Hospital records of premature babies with and without Tween 80 supplement in the diet have not revealed obvious indications of ill effects. However, as long as a systematic survey is missing in regard to premature tolerance of Tween 80, observations of a toxicity for embryonic tissues, even in an unrelated species, may make it advisable to follow critically the

‡ This figure is taken from R. Rugh, *Exp. Embryology*, 1948, p.450.

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