labeling indices, and a reduction in DNA synthesis. It was suggested that pershaps the burst of incorporation of H^3TDR within the first 12 hours was due to an initial depletion of endogenous DNA precursors. Similar results were observed in the intact femora exposed to 100% O_2, since at 8 and 12 hours the labeling was higher than that observed at 30 hours and also in the control group subjected to air.

Summary. It is concluded from the present H^3TDR autoradiographic study of mouse femora that 5% oxygen leads to suppression of proliferation in an intact periosteum and cessation of proliferation when the periosteum is injured. The deleterious effects of 100% oxygen on the proliferation of cells are not realized at the fracture site, perhaps because of the protection afforded by the existing anoxia. In the intact limb there is evidence to show an early deleterious effect of 100% oxygen.

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1. Astrand, P., Christensen, E. H., in Oxygen in the Animal Organism, F. Dickens, E. Neil, eds., Pergamon Press, New York, 1963, p295.
2. Bacq, A. M., Alexander, P., ibid., p509.
3. Bean, J. W., ibid., p455.
4. Bergofsky, E. H., Jacobson, J. H., Fishman, A. P., J. Clin. Invest., 1962, v41, 1971.
5. Chance, B., Fed. Proc., 1957, v16, 671.
6. Chance, B., Cohen, P., Jobsis, F., Schoner, B., Science, 1962, v137, 499.
7. Drew, R. M., Painter, R. B., Feinendegen, L. E., Exp. Cell Res., 1964, v36, 297.
8. Eastabrook, R. W., Hollowinsky, A., J. Biophys. Biochem. Cytol., 1961, v9, 19.
9. Foster, R. W., Ann. N. Y. Acad. Sci., 1965, v117, 730.
10. Gerschman, R., in Oxygen in the Animal Organism, F. Dickens, E. Neil, eds., Pergamon Press, New York, 1963, p475.
11. Goldhaber, P., The Parathyroids, C. C Thomas, Springfield, Ill., 1961, p243.
12. Gray, L. H., Scott, O. C. A., in Oxygen in the Animal Organism, F. Dickens, E. Neil, eds., Pergamon Press, New York, 1963, p537.
13. Green, D. E., Hatefi, Y., Science, 1961, v133,
14. Haugaard, N., Ann. N. Y. Acad. Sci., 1965, v117, 736.
15. Jobsis, F. F., in Handbook of Physiology, Am. Physiol. Soc., Washington, D.C., 1964, v1, 111.
16. Thomson, J. F., in Radiation Protection in Mammals, Rheinhold Pub., New York, 1962, p45.
17. Tonna, E. A., J. Biophys. Biochem. Cytol., 1961, v9, 813.
18. Tonna, E. A., Cronkite, E. P., J. Bone & Joint Surg., 1961, v43A, 352.
19. ———, Lab. Invest., 1962, v11, 455.
20. ———, J. Bone & Joint Surg., 1962, v44A, 1557.
21. ———, J. Cell Biol., 1964, v23, 79.

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Suppression of Toxoplasmin Skin Reactivity in Sensitized Guinea Pigs by Multiple Inoculations. (31802)

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An earlier publication described a laboratory procedure for determining the potency of toxoplasmins used as intradermal test antigens for diagnosis of toxoplasmosis(1). Subsequent experiments have demonstrated an inhibitory effect on skin reactions when 2 or more toxoplasmin preparations or dilutions of the same preparation are tested simultaneously in the same animal. The results presented here show that strongly reactive antigens may suppress lesser reactive antigens and that mutual suppression may occur between preparations of approximately equal potency.

Materials and methods. Sensitization of guinea pigs. Toxoplasmin prepared from

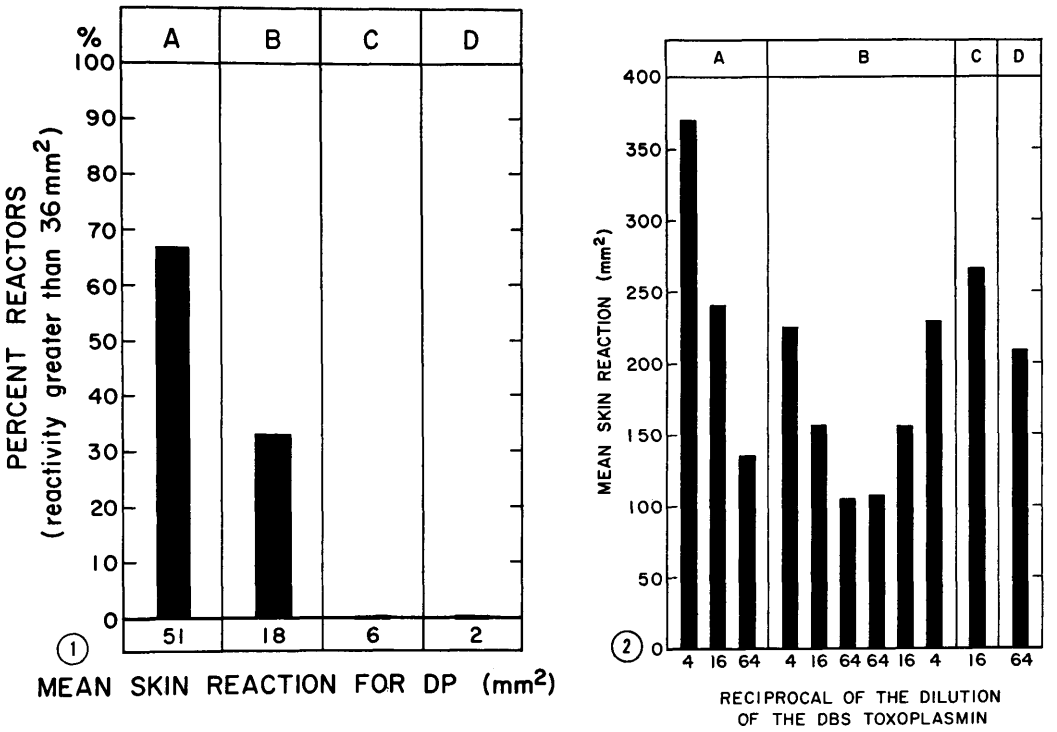

FIG. 1. Suppression of DP toxoplasmin reactivity by DBS toxoplasmin. Animals tested with: A. DP alone; B. DP and 1:64 dilution DBS; C. DP and 1:16 dilution DBS; D. DP and 2 injections of DBS dilutions 1:4, 1:16, 1:64. 100% of animals responded to DBS toxoplasmin with skin reactions greater than 36 mm².

FIG. 2. Suppression of DBS toxoplasmin reactivity by DBS toxoplasmin. Animals tested with: A. 1 injection of DBS dilutions 1:4, 1:16, 1:64; B. 2 injections each, one on each side of animal, of 1:4, 1:16, 1:64 dilution of DBS; C. 1:16 dilution of DBS, D. 1:64 dilution of DBS.

Toxoplasma gondii grown in monolayers of the LLC-MK* strain of monkey kidney cells (2) mixed in complete Freund's adjuvant containing killed BCG was used to sensitize guinea pigs. Four-tenths ml of the emulsified antigen was injected subcutaneously into groups of 6-10 female, Hartley strain guinea pigs, each weighing 225-275 g. Details of the procedure have been described(1). Intradermal injection induced equal sensitization, but due to ulceration the subcutaneous route was used in all but one experiment.

Skin test antigens. Three toxoplasmin antigens were prepared from the peritoneal fluid of mice infected with the RH strain of *Toxoplasma gondii* as follows: (a) DBS toxoplasmin was extracted by alternate freeze-thaw cycles of toxoplasmata harvested from infected peritoneal fluid; (b) NIAID toxoplasmin† was prepared by distilled water lysis of toxoplasmata after washing the parasites in saline and separation from other peritoneal fluid elements by sintered glass filtration(3); (c) DP toxoplasmin was prepared like DBS toxoplasmin by a third laboratory and had been diluted 1:100 before receipt.

Control material was a saline extract of ground spleens from normal, healthy mice that had been subjected to 5 freeze-thaw cycles(1).

Skin tests. Intradermal injections of 0.1 ml toxoplasmin were made in guinea pigs 6 weeks after the sensitizing injection. Activity was expressed as the product of the greater and lesser diameters of erythema at 24 hours. A 36 mm² (d × d) skin reaction

* Kindly supplied by Mr. Robert Grubbs, DBS.

† Kindly supplied by Dr. Leon Jacobs, DBS, and Mr. Milford Lunde, NIAID.

was the criterion for the lower limit of a positive test reaction to toxoplasmin. The mean of the reactions induced by the control extract diluted 1:2, 1:4, and 1:16 was 15 mm^2 and diluted 1:64 and 1:100 was 8 mm^2. These reactions were paler than those induced by toxoplasmin.

Results. The suppression of DP toxoplasmin reactions by DBS toxoplasmin was directly related to the amount of DBS antigen injected (Fig. 1). DP toxoplasmin tested alone induced positive reactions (36 mm^2) in 4 of 6 animals (67%) and the mean reaction was 51 mm^2 (Group A). When a 1:64 dilution of DBS toxoplasmin was tested simultaneously with DP toxoplasmin in another group of 6 animals, only two showed a positive reaction and the mean reaction was reduced to 18 mm^2 (Group B). The mean reaction to the DBS toxoplasmin was 208 mm^2. When the amount of DBS toxoplasmin was increased to 1:16, no animal showed a positive reaction for DP toxoplasmin and the mean reaction was only 6 mm^2 (Group C). The mean reaction to the DBS toxoplasmin was 266 mm^2. When DBS toxoplasmin diluted 1:4, 1:16, 1:64 was injected bilaterally for a total of 6 injection sites with an additional site for the test DP toxoplasmin, the reaction to the latter was almost completely suppressed (Group D). The mean reactions to DBS toxoplasmin dilutions of 1:4, 1:16, and 1:64 were 354, 239 and 149 mm^2, respectively.

The suppression of DBS toxoplasmin reactions by itself is shown in Fig. 2. Dilutions of 1:4, 1:16, and 1:64 of DBS toxoplasmin tested simultaneously on the same animal induced mean skin reactions of 370, 240, 135 mm^2, respectively (Group A). However, when 2 injections of each of the 3 dilutions were made, one series on each side of the animal, there was mutual suppression in the development of the skin reactions: 39% for the 1:4 dilution, 36% for the 1:16 dilution, and 22% for the 1:64 dilution (Group B). On the other hand, when the 1:16 and the 1:64 dilutions were tested singly (Groups C and D), the reactions exceeded those when 1:4, 1:16, and 1:64 dilutions were tested on the same animal (Group A). The 1:16 dilution reactions were 11% larger and the 1:64 dilution reactions were 54% larger.

Mutual suppression of DP, DBS and NIAID toxoplasmin reactions was demonstrated in another set of experiments in which 5 groups of 10 sensitized guinea pigs each were used. The toxoplasmins were injected either alone or together in an animal (Fig. 3). Although 100% of the animals responded to DBS and NIAID toxoplasmin when tested alone, Groups A and B, respectively, only 70% of the animals responded to either of these preparations when tested simultaneously with DP in the same animal (Groups D and E, respectively). Only 60% of the animals responded when tested with DP alone (Group C) and an even lower frequency of reactivity was noted when animals were tested with DP along with either DBS or NIAID, 40% and 30%, respectively, for DP (Groups D and E).

Discussion. The results of this study illustrate the ability of strongly reactive doses of toxoplasmin skin test antigens to suppress weaker reactive doses when tested simultaneously in the same animal. This finding points up the need in the standardization of toxoplasmin skin test antigen to test doses of preparations of approximate potency. Although mutual suppression occurred when 2 series of dilutions of a toxoplasmin preparation were tested in the same animal, the degree of suppression was found to be distributed equally between the two test series and the relative reactivity of the antigens remained the same. However, in the assay of an unknown antigen in terms of a reference antigen, it will be necessary to select the proper dilution prior to comparison with the reference antigen.

Another factor to consider is the variability of the guinea pigs. Ideally, animals should be preselected on the basis of equivalent reactivity to a given skin test dose of antigen. This presents a problem in testing toxoplasmin because the animals may become sensitive to the antigens of the cells from which the toxoplasmin was derived(1). In view of this one may either use a large number of animals for standardization of

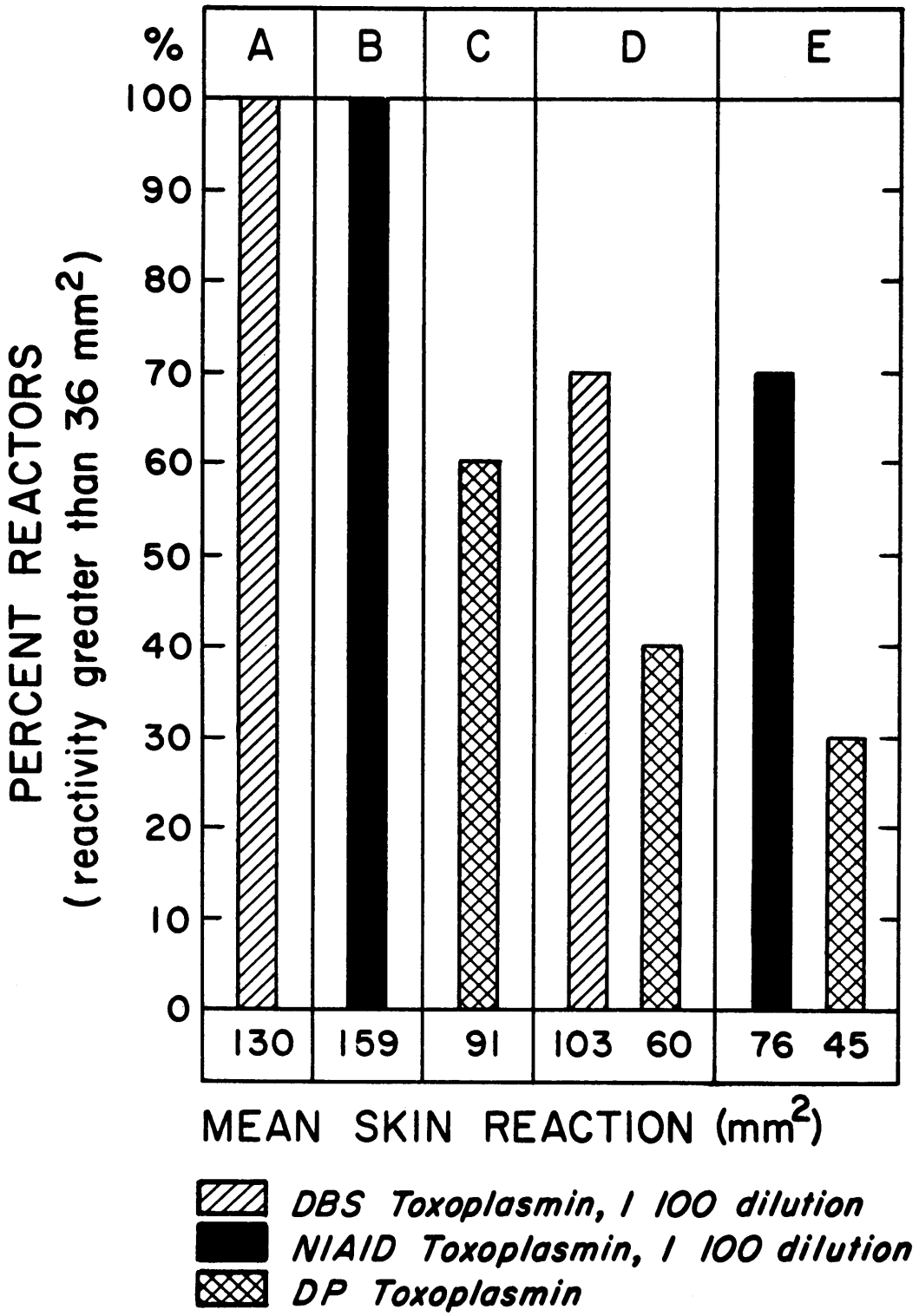

FIG. 3. Mutual suppression of DP, DBS and NIAID toxoplasmin reactivity.

skin test antigens or preselect animals of equivalent reactivity. In the latter case, 3 toxoplasmin preparations, each derived from a heterologous cell source, would be needed; one to be used to sensitize the animals, a second to select animals and a third to be standardized.

Whether the nature of the toxoplasmin suppression is due to the dissipation of specifically sensitized cells or to the dissipation of nonspecific cellular elements or both has not been resolved.

Suppression of the tuberculin skin test reaction by tuberculin (unpublished observations of Baer, H. and Kolb, R. W.) and unrelated agents, such as viruses, has been demonstrated(4,5,6). Whether or not the basis for suppression of reactivity of toxoplasmin and the other agents is analogous is not known.

Summary. Suppression of the skin reactivity to toxoplasmin by simultaneous injection of larger amounts of the antigen in the same sensitized guinea pigs was demonstrated by fewer animals reacting to the lesser amount of toxoplasmin and a decrease in the mean skin reaction as compared with the reaction when the toxoplasmin was injected alone.

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1. Chaparas, S. D., Fuller, V. J., Kolb, R. W., Proc. Soc. Exp. Biol. and Med., 1966, v121, 734.

2. Evans, V. J., Kerr, H. A., McQuilkin, W. T., Earle, W. R., Hull, R. N., Am. J. Hyg., 1959, v70, 297.

3. Lunde, M. N., Jacobs, L., J. Parasitol., 1964, v50, 49.

4. Pepys, J., Am. Rev. Tuberc., 1955, v71, 49.

5. Arnason, B. G., Waksman, B. H., Adv. Tuberc. Res., 1964, v13, 1.

6. Brody, J. A., Overfield, T., Hammes, L. M., New Eng. J. Med., 1964, v271, 1294.

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Local Electrographic Responses to Intrahippocampal d-Tubocurarine and Related Neuromuscular Blocking Agents.* (31803)

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When injected into the cerebral ventricles, d-tubocurarine (dTC) markedly excited the central nervous system, causing extensive autonomic changes and convulsions(1). By selective perfusion of the individual ventricles, the actions of dTC were restricted more specifically to structures immediately lining the ventricle to produce tremors, myoclonic jerks or EEG discharges(2,3). On further analysis, the abnormal electrographic activities were shown to originate primarily from an action on the hippocampus(4,5). Comparable spike discharges were more readily developed by microinjecting the drug directly into the hippocampus(6,7). Our research was primarily structured to analyze the local electrographic paterns of excitation induced by dTC microinjected into the hippocampus. To characterize this activity further, the study was expanded to include other neuromuscular blocking agents. We hoped also to characterize the local mechanisms associated with hippocampal effects of dTC and if possible, to relate these to the cholinergic receptor mechanisms which have been defined pharmacologically at the neuromuscular junction.

Materials and methods. Acute experiments were carried out on 41 male adult cats (2.4-4.0 kg) while they were lightly anesthetized with dial-urethane, immobilized with either decamethonium or gallamine triethiodide (Flaxedil) and respirated. These animals had been prepared previously under ether anesthesia during which bipolar recording electrodes were placed stereotaxically in several key brain areas. Also, at this time, a microinjection cannula-electrode assembly ("Injec-

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