16712

Response of Normal and Malignant Lymphoid Tissue to Non Specific Tissue Damage.*

ALLAN D. BASS AND MURIEL FEIGELSON.

From the Department of Pharmacology, College of Medicine, Syracuse University, Syracuse, N. Y.

In the evaluation of possible therapeutic agents in experimental lymphoma it has become apparent that drugs producing lymphoid tissue atrophy may do so either by direct action on the lymphocyte itself or an indirect action initiating an "alarm" reaction. Bass and Freeman[1] using 95% ethyl alcohol as a stimulous have shown that lymphoma 6C3HED responds in a manner similar to that of normal lymphoid tissue. That the alcohol itself was not a lympholytic agent was evident, as larger amounts of ethyl alcohol diluted to 19% concentration produced no lympholytic effects. Certain agents, such as nitrogen mustard and urethane, produce atrophy of lymphoid tissue through two possible mechanisms: (a) initiation of an alarm reaction[2,3] and (b) a direct action of the drug upon the lymphoid organs. The latter effect, which is the most important one, has been demonstrated by administration of these agents to adrenalectomized mice bearing lymphoid tumors.[4] It is, therefore, apparent that screening technics for new lympholytic agents must take into consideration both the direct and indirect effect upon lymphoid tissue. The present study was undertaken to establish further relationship of malignant tissue response to the administration of established stress stimuli. Those selected for the study were 40% glucose[5] and 4% formaldehyde.[6] Since it has been reported by Selye,[7] using the rat as his experimental animal, that fasting is either an alarming stimulus in itself or potentiates alarming stimuli, the effect of fasting in mice was also included in this study.

Materials and methods. The pure strain C3H male mice employed in this study were obtained from Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. The C3H F_1 hybrids of mixed sexes were bred in this laboratory. All animals were maintained on Purina dog chow and allowed free access to water at all times.

Transplanted lymphoma, 6C3HED, was employed in this investigation. A small particle of tumor tissue was implanted subcutaneously into the right axillary region of each animal by means of a 15 gauge needle. Procedures initiating alarming stimuli were instituted when tumors had become definitely measurable (seven days after implantation in pure strain C3H mice; seven to eleven days after implantation in C3H F_1 hybrid mice).

Intraperitoneal injections of 95% ethyl alcohol, U. S. P., were given to tumor animals in daily doses of 0.02 ml per mouse for 2 days. Animals used for spleen and thymus weight studies received 1.5 ml per kg at the same intervals. An aqueous solution of 40% glucose was administered intraperitoneally in doses of 150 ml per kg of body weight daily for 2 days. Subcutaneous injections of 4% formaldehyde were given twice daily for two days in doses of 5 ml per kg of body weight. Agents repeated within one day were given

* This investigation was supported by a research grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

1 Bass, A. D., and Freeman, M. L. H., *Science*, 1948, **107**, 114.

2 Ludewig, S., and Chanutin, A., *Endocrinol.*, 1946, **38**, 376.

3 Karnofsky, D., Graef, I., and Smith, H. W., *Am. J. Path.*, 1948, **24**, 275.

4 Bass, A. D., and Feigelson, M., *Cancer Research*, in press.

5 Reiss, M., Macleod, L., Golla, Y., *J. Endocrinol.*, 1943, **3**, 292.

6 Selye, H., *Brit. J. Exp. Path.*, 1936, **17**, 234.

7 Selye, H., *J. Clin. Endocrinol.*, 1946, **6**, 117.

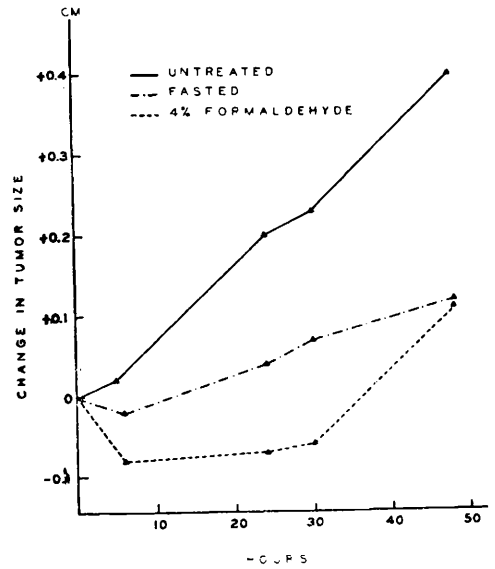

FIG. 1.

C3H hybrid mice were used. Number of animals employed: untreated 6, fasted 9, formaldehyde 10. Therapy was started at zero time.

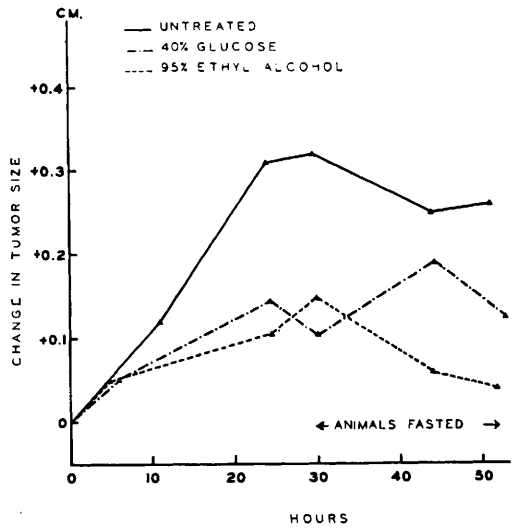

FIG. 2.

C3H mice were used. Number of animals employed: untreated 11, 95% alcohol 11, 40 glucose 12. Treatment was begun at zero time.

6 hours apart. Fasted animals were deprived of all food but were given water *ad libitum*. Tumor measurements were made twice daily. The longitudinal and transverse diameters of each tumor were measured with calipers and the mean dimension (one half of the length plus the width expressed in centimeters) was arbitrarily used as the tumor size. Animals used for organ weight studies were killed with ether 48 hours after therapy was initiated. Spleens and thymuses were carefully dissected out and weighed immediately on a Roller-Smith micro torsion balance.

Results. The effect of subcutaneous injection of 4% formaldehyde and of a 48 hour fast upon the tumor growth in C3H hybrid mice of mixed sexes is illustrated in Fig. I. It is clearly shown that the tumor growth curve of these treated animals is definitely altered from that of the untreated animals. Formaldehyde administration induced a definite regression whereas fasting induced only an inhibition of tumor growth. Fig. 2 shows the effect of two other alarm stimuli (95% ethyl alcohol administered intraperitoneally and 40% glucose administered intraperitoneally) on the change in tumor size in pure strain C3H male mice. Beginning 30 hours after the initial stimuli all animals were fasted for a period of 24 hours. Both stimuli (ethyl alcohol and glucose) are shown to inhibit tumor growth during the initial 30 hour period. Fasting under these conditions definitely potentiated the inhibiting effects of 95% ethyl alcohol and resulted in tumor regression in untreated animals. A less definite effect on the glucose treated animals was observed.

Table I demonstrates the effect of 95% ethyl alcohol, 4% formaldehyde, 40% glucose and a 48-hour fast on splenic and thymic wet weights in C3H F_1 hybrid mice of mixed sexes. Formaldehyde produced a statistically significant reduction in both spleen and thymus weights. Thymus weights of ethyl alcohol-treated animals and of fasted animals were significantly less than those of the untreated controls. Although all of the means of organ weights were not statistically significant, the mean spleen and thymus weights of all treated animals were lower than the untreated controls.

Summary. Further evidence has been added to indicate that ethyl alcohol is an alarming stimulus. We have shown that the non-specific stimuli, 4% formaldehyde and 40% glucose, in unadrenalectomized mice

TABLE I.
C3H Hybrids (Mixed Sexes) Sacrificed 48 Hours After Initial Therapy.

Treatment	Dose schedule	No. animals	Organ weights, g per 100 g body wt: Mean spleen wt ± S.E.	Mean thymus wt ± S.E.
None	—	15	1.348 ± 0.0802	0.153 ± 0.0194
95% ethyl alcohol	1.5 ml/kg daily for 2 days	7	1.050 ± 0.1259	0.051 ± 0.0101*
4% formaldehyde	5 ml/kg 2 times daily for 2 days	9	0.605 ± 0.742*	0.050 ± 0.0056*
40% glucose	15 ml/kg daily for 2 days	10	1.055 ± 0.1187	0.112 ± 0.0152
48-hour fast	—	9	1.138 ± 0.1031	0.107 ± 0.0147*

* Upon statistical analysis weights are significantly smaller than untreated controls.

temporarily inhibit growth or produce temporary regression of the malignant lymphoid tumor 6C3HED. A similar effect is produced by a 48-hour fast. Fasting begun 30 hours after the initial stimulus gives an additional "lympholytic" effect.

It has been shown that stimuli which are known to produce an alarm reaction with resulting atrophy of the normal thymus and normal spleen produce a similar type of reaction in tissue composed of malignant lymphocytes.

16713

Human Skin Grafted Upon the Chorioallantois of the Chick Embryo for Virus Cultivation.*

HARVEY BLANK,† LEWIS L. CORIELL,‡ AND T. F. MCNAIR SCOTT.
(Introduced by Werner Henle.)

From The Children's Hospital of Philadelphia, and the Departments of Dermatology and Pediatrics, University of Pennsylvania, School of Medicine, Philadelphia.

Goodpasture, Anderson, and Douglas have described a technic for grafting human skin onto the chorioallantoic membrane of embryonated eggs.[1,2] They demonstrated that viruses such as herpes simplex and vaccinia, which multiply readily in the normal embryonated egg, would also grow in the grafted skin with the appearance of characteristic inclusion bodies in the epithelial cells. In addition, by using this technic they recorded an experiment in which they were able to grow herpes zoster on a single occasion.[3] They also indicated the value of such heterologous skin grafting in immunologic studies, by demonstrating that skin taken from chickens immune to fowl pox would lose its immunity after being grafted onto the chorioallantois.[4] It is assumed that the immune bodies are diluted out of the skin as it grows in the egg.

The obvious usefulness of vascularized, morphologically distinct human skin, actively growing in a sterile host that does not produce antibodies of its own, prompted the

* This study was supported by grants from The Lederle Laboratories Division, American Cyanamid Company, the U. S. Public Health Service, and the National Foundation for Infantile Paralysis.

† Fellow in the Medical Sciences, National Research Council, during part of this study.

‡ Senior Fellow in Virus Research, National Research Council.

1 Goodpasture, E. W., Douglas, B., and Anderson, K., *J. Exp. Med.*, 1938, **68**, 891.

2 Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1942, **18**, 563.

3 Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1944, **20**, 447.

4 Goodpasture, E. W., and Anderson, K., *Arch. Path.*, 1940, **30**, 212.