The antigenicity of the supernatants was tested by the passive hemagglutination method (HA)[10] and by the CASONI intradermo reaction test (IDR)[11]. The results (Table I) indicated that the precipitate appearing in presence of phosphate buffer was accompanied by a diminution in the antigenic activity of HF.

Concentrated HF dialysed against *Tris*-HCl 0.01 *M*, pH 7.4 buffer was placed on a 20 · 1.5 cm column packed with DEAE-Sephadex A 50 medium, equilibrated with the *Tris*-HCl buffer. Elution was performed with a 0.01–0.4 *M* NaCl gradient. The chromatographic fractions were pooled as indicated in Figure 1, concentrated and tested for HA and CASONI reaction.

Maximal antigenic activity in the HA test was found in fraction VII and in the IDR in fraction IV as summarized in Table II. HF fraction IV and fraction VII were analysed by electrophoresis in polyacrilamide gel[12] and by inmuncelectrophoresis[13] using rabbit antisera against human serum, ovine serum, human IgG, and IgA. Gel electrophoresis revealed 4 components in fraction IV and only one component in fraction VII (Figure 2). The latter has a mobility similar to albumin.

By inmunoelectrophoresis it was shown that fraction IV contained IgA. Further characterization of the components present in the antigenic fractions is in progress.

Resumen. Utilizando columna cromatográfica con DEAE-Sephadex A-50 medium, pudimos aislar dos importantes fracciones antigénicas a partir de liquido hidático. Una (IV) con gran actividad en reacción intradermica y otra (VII), altamente purificada con gran actividad en hemaglutinación pasiva.

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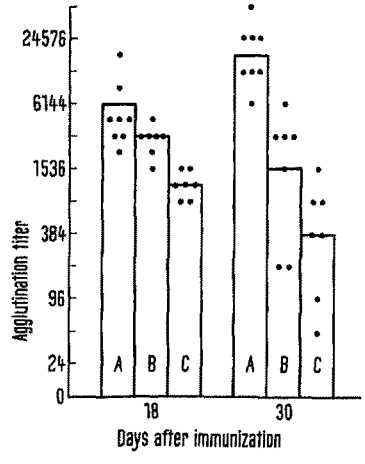
Potentiation of the Immunosuppressive Effects of Cytoxan by Hyperbaric Oxygen

Previous work from this laboratory[1] has shown that a regimen of long-term intermittent hyperbaric oxygenation (HPO) exerts a protective effect on mice infected with a leukemogenic virus. Also, this HPO treatment has been demonstrated to cause a transient decrease in size of thymuses and spleens of otherwise normal animals[2]. These observations suggested that the effect of HPO might be on proliferation of lymphoid tissue and that hyperbaric oxygenation, alone, or in conjunction with other agents, might exert a depressing effect on the immune response. In subsequent experiments it was noted that circulating antibody titers to sheep red blood cells (SRBC) were not significantly affected by HPO in normal or cytoxan-treated mice[3]. These findings did not exclude the possibilities of demonstrable oxygen effects on the antibody response of animals immunized with different types of antigenic stimuli. The results of such a study employing bovine serum albumin (BSA) in complete Freund's adjuvant as the antigenic system are described in the present report.

The HPO regimen employed has been described in detail[1,2] and, briefly, was as follows. Five-week-old female Balb/c/jax mice were subjected to 97% oxygen in 3.4 atmospheres absolute pressure for 30 min periods, twice daily, 5 days per week. Temperature inside the chamber was maintained at 70 °F ± 3° by means of a thermostatically controlled water jacket surrounding the chamber. The hyperbaric oxygen regimen was started on the day of immunization, several hours after cytoxan injection, and was continued throughout the experiment. No discomfort or convulsive behavior was observed either during or following oxygen treatment. Mice were immunized by a single i.p. injection of 0.25 ml of a complete Freund's adjuvant (Difco Laboratories, Detroit) emulsion containing 1.25 mg of bovine serum albumin (Fraction V, crystalline, Pentex). Four hours after immunization, cytoxan (Cyclophosphamide, Meade-Johnson) was administered as a single i.p. injection of 80 mg/kg weight. Antibody titers were determined on two-fold serial dilutions of individual mouse sera using the tanned, antigen-coated erythrocyte method of STAVITSKY[4].

As can be seen in the Figure, the suppressive effects of cytoxan were significantly augmented by hyperbaric oxygen treatment, the greatest suppression appearing after 30 days, when control antibody levels were at a maximum. Hyperbaric oxygen treatment of immunized mice not receiving cytoxan resulted in titer values



Agglutinin responses to BSA in complete Freund's adjuvant, expressed as reciprocal titers, in Balb/c/jax mice. Each circle (●) represents serum from a single animal, while bar heights represent mean titers for each group. (A) immunized only, (B) immunized plus cytoxan, (C) immunized plus cytoxan plus HPO.

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essentially identical to those shown for the immunized-only mice (group A). Toxicity in terms of deaths was the same for both cytoxan and the cytoxan plus HPO treated mice, namely, 1 death out of 8 experimental animals.

Our previous studies[5-7] have suggested that mycobacterial adjuvant functions to enhance the transformation of small lymphocytes to immunopotential stem cells, thereby providing larger numbers of progenitor cells capable of responding to a primary immune stimulus. This adjuvant effect has been observed to continue over a period of 30 days or more in the mouse following a single injection of adjuvant[6]. It might be speculated that immunization with heterologous erythrocytes[3], on the other hand, influences the differentiation and proliferation of the available stem cell pool; but any small lymphocyte transformation which may occur does so after the primary inductive role of this antigen is terminated, and is thus largely a passive result of the need to replenish the stem cell pool size. It would appear then, that the potentiating action of HPO on cytoxan induced immunosuppression in adjuvant-BSA immunized mice, and absent in SRBC immunized animals, is directed towards interference with the small lymphocyte to stem cell transformation. Since stimulation of this type cell transformation may also be fundamental to leukemic processes[7], an analogy between the high pressure oxygen effect in depressing an adjuvant-immunization system and that in retarding leukemogenesis[1] would appear consistent with this interpretation[8].

Zusammenfassung. Die intermittierende Anwendung hyperbarischen Sauerstoffs erhöht die die Antikörper unterdrückende Wirkung von Cytoxan bei Mäusen, die vorher mit Ochsenserum-Albumin und Freunds Adjuvans immunisiert worden waren. Der Wirkungsmechanismus des Sauerstoffs wird als Verhinderung der Transformation von kleinen Lymphocyten in immunbiologisch aktive Stammzellen gedeutet.

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Effect of Hypothalamic Lesions on MSH Content of the Intermediate Lobe of the Pituitary Gland in the Rat

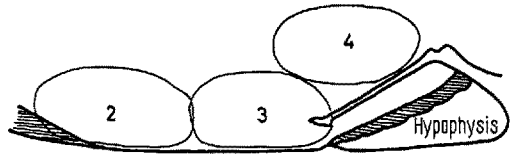
It has been reported that, as in amphibians, interruption of the hypothalamo-hypophysial connections in mammals results in a hypertrophy of the intermediate lobe of the pituitary gland[1]. This suggests that the central nervous system may exert a mainly inhibitory influence on the secretion of melanophore-stimulating hormone (MSH).

We have produced lesions in the hypothalamus of the rat in order to investigate whether the amount of MSH in the intermediate lobe would be affected by this procedure.

Experimental. Female albino rats, weighing between 120 and 140 g, were placed in a stereotaxic instrument and a radio-frequency coagulation was performed in several parts of the hypothalamus. Control animals were sham-operated, but no electrode was introduced into the brain. Each experiment consisted of 4 groups of animals, receiving: (1) a sham-operation; (2) a midline lesion in the region between optic chiasm and median eminence; (3) a midline lesion destroying the posterior part of the median eminence and the hypophysial stalk area; (4) a bilateral lesion in the mammillary bodies. The position of these lesions is illustrated in the Figure.

After 1 week the rats were decapitated. The posterior lobe (including pars intermedia and pars nervosa) was carefully separated from the anterior lobe, and homogenized in 0.5 ml of 0.1 *N* HCl. The acid extract was then diluted with Ringer solution, and the MSH concentration determined in vitro on colour change in pieces of skin of the lizard *Anolis carolinensis*[2]. This sensitive assay can detect a concentration of about 0.1 ng/ml of synthetic α-MSH. As a reference standard synthetic α-MSH has been used, so that the contents could be expressed as μg α-MSH.

Each experiment was repeated 5 times, and the results were pooled according to the site of the lesion afterwards



Sagittal section through the hypothalamus, indicating the areas common to the lesions within a given experimental group (2, 3, 4).

MSH content of pituitaries from rats bearing lesions at several locations in the hypothalamus

Group	No. of rats	Lesion site	MSH content μg/posterior lobe	Difference from controls
1	27	No lesion	1.1 ± 0.1	–
2	36	Anterior-basal	0.7 ± 0.1	$P < 0.05$
3	23	Stalk region	0.9 ± 0.2	Non significant
4	15	Posterior-dorsal	1.9 ± 0.4	$P = 0.08$

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