Effect of Continued Treatment with Cortisol on Thymidine Incorporation Into Mouse Lymphatic Tissue Nucleic Acid.* (31784)

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The reduction in lymphatic tissue mass produced by cortisol is a well known phenomenon. This reduction is brought about by at least 4 observable effects of cortisol: the actual destruction of mature lymphocytes (1,2,3), the increased rate of maturation of immature lymphocytes with their subsequent destruction as mature cells(1,2,3), cessation of mitosis(1), and inhibition of metabolic processes within the cells(4,5,6). One of the principal metabolic effects of cortisol is inhibition of nucleic acid synthesis(7,8,9). All of these responses can be elicited by a single treatment with cortisol.

It has been shown by other investigators in this laboratory that continued treatment with cortisol produced an increase in the lymphocytes' ability to inactivate the hormone by transforming it to compounds which lack these biological effects(10,11). The problem under investigation is that, if these cells have an increased ability to inactivate cortisol is their ability to synthesize DNA also altered?

The study reported here was undertaken to examine the daily effects of prolonged cortisol treatment on DNA synthesis following daily injections of hormone for up to 5 days.

Materials and methods. Male mice of the CBA strain 10-14 weeks of age, were used as the experimental animals. The animals in the control group each received 0.25 ml of sterile saline† a day for up to 5 days. Each of the cortisol treated animals received 1 mg of hydrocortisone acetate‡ in 0.25 ml of sterile saline a day for up to 5 days. A group of non-injected controls was used for comparison to saline controls and cortisol treated animals. Six hours after each injection the saline controls and cortisol treated animals were each given 1 μCi of thymidine-2-^{14}C intraperitoneally (SA 1 μCi = 0.0327 μMoles). Thirty minutes after the thymidine administration the animals were sacrificed. Inguinal and axillary lymph nodes, spleens, and thymi were removed, cleaned of connective tissue, weighed and immediately frozen until extractions of nucleic acids were performed. The tissues of 5 animals were pooled and the organ weights were determined. The organs were then extracted for nucleic acids. Body weights were taken just prior to sacrifice.

The nucleic acids were extracted using the method of Hecht and Potter(12). The amounts of nucleic acids in each sample were quantitated by ultraviolet absorption at 258.5 mμ. Sigma DNA sodium salt type I from calf thymus was used as a standard. The amount of radioactivity in each sample was determined in a liquid scintillation spectrometer. Efficiencies of the counting were determined by using internal standards. The data are presented as dpm/milligram of total nucleic acid. No attempt was made to separate DNA and RNA.

There was a total of 4 control groups and 3 experimental groups. Statistical analysis of the data was performed using an analysis of variance, single classification completely randomized design together with a "Q" test to determine between which groups differences occur.§ Statistical computations were done on an IBM 7040 computer.

Results. Fig. 1 graphically illustrates the response in organ weights of the spleen, thymi and lymph nodes to continued treatment with cortisol. The weight of the spleen in the treated animals after one injection of cortisol was decreased from the saline controls. This decrease continued until the last day of

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† Obtained from Cutter Laboratories.

‡ Generously supplied by Dr. Elmer Alpert, Merck Sharp & Dohme.

§ Federer, W. T., Experimental Design, Macmillan Co., New York, 1955.

treatment. The weights of thymi of cortisol were not different and lymph nodes following 1 mg from their saline injected controls,

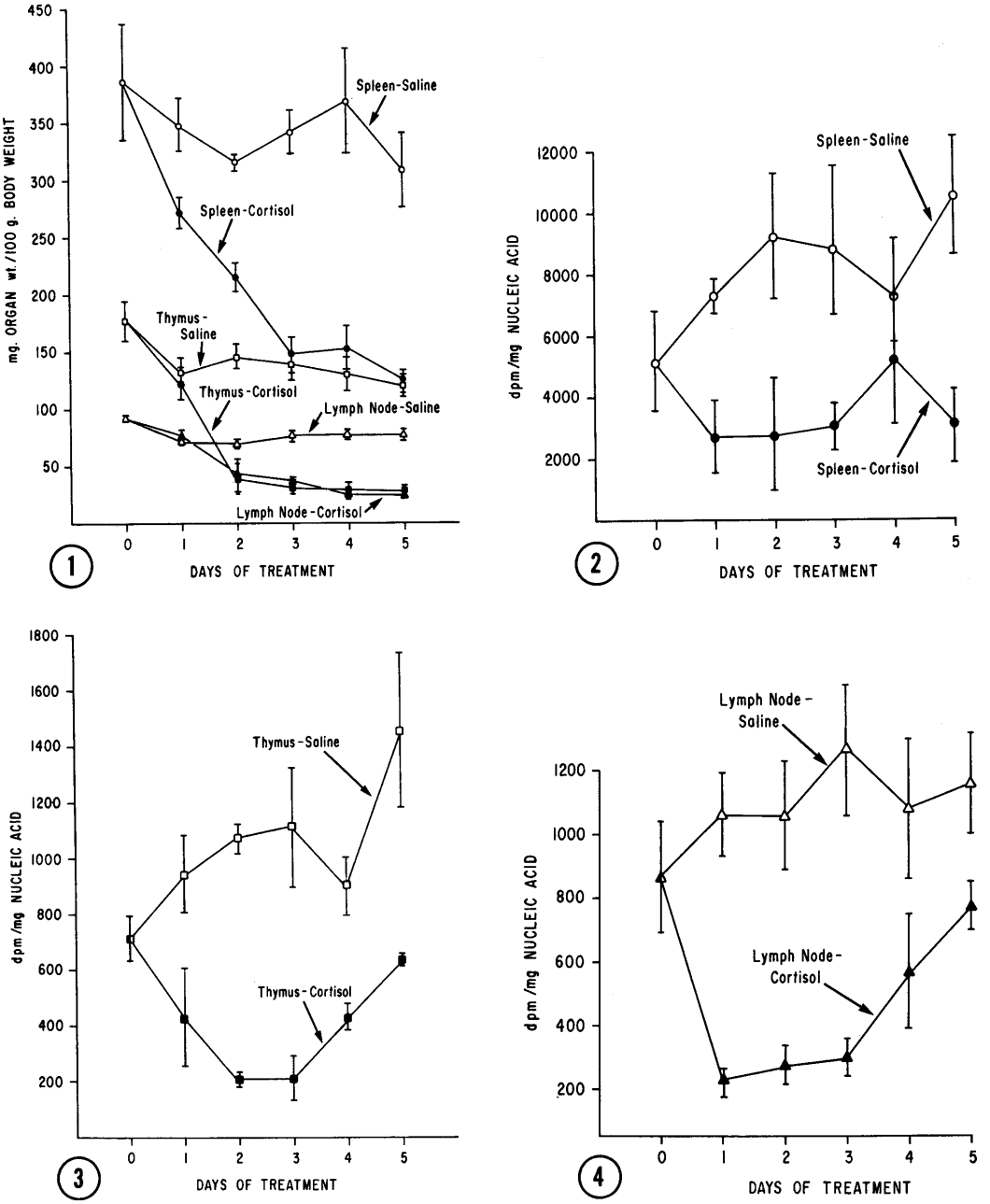

FIG. 1. Effect of daily injection of 1 mg cortisol acetate or saline on organ weights of spleens, thymi, and lymph nodes. (Mean ± 1 Standard Error).

FIG. 2. Effect of daily injections of 1 mg cortisol acetate or saline on thymidine-2-^{14}C incorporation into nucleic acids of mouse spleens. (Mean ± 1 Standard Error).

FIG. 3. Effects of daily injections of 1 mg cortisol acetate or saline on thymidine-2-^{14}C into nucleic acids of mouse thymi. (Mean ± 1 Standard Error).

FIG. 4. Effects of daily injections of 1 mg of cortisol or saline on thymidine-2-^{14}C incorporation into nucleic acids of mouse lymph nodes. (Mean ± 1 Standard Error).

but were decreased after 2, 3, 4 and 5 mg of cortisol. There appeared to be no furthr decrease in organ weights after the second cortisol injection. Injection of either saline or cortisol caused a small decrease in organ weights from the non-injected controls.

The data plotted in Fig. 2, 3, and 4 show the amounts of thymidine-2-^{14}C incorporated into total nucleic acids of spleen, thymi, and lymph nodes following treatment with 1 mg of cortisol acetate a day for up to 5 days and with saline for the same time period.

The spleen (Fig. 2) shows a decrease in thymidine incorporation following 1 mg of cortisol. There was no further decrease with continued hormone treatment. Saline treatment produced an increase in thymidine incorporation over non-injected controls. There are statistically significant differences between cortisol treatment and saline treatment on days 2, 3, and 5. There are no differences within the saline or cortisol treated groups.

The data from the thymus (Fig. 3) and lymph nodes (Fig. 4) show a different response than the data from the spleen. Both organs had an increase in thymidine incorporation following saline treatment. The response of the thymus to saline treatment was more variable than in the lymph nodes. Cortisol treatment, however, produced a decrease in thymidine incorporation in thymus and lymph nodes following 1, 2, and 3 days of treatment. There was an increase in nucleic acid specific activity (dpm/mg NA) after 4 and 5 days of treatment. In the lymph nodes this increase is large enough so that after 5 days the difference between saline treated and cortisol treated groups is no longer statistically significant. This however was not true in the thymus due to the large increase in the saline treated group at 5 days. The differences in the thymus and lymph nodes between saline treatment and cortisol treatment are statistically significant at 1, 2, and 3 days post treatment.

In the lymph nodes (Fig. 4) the incorporation of thymidine after 5 days of treatment is significantly greater than it was after 3 days of treatment. There are no significant differences within groups in the saline treated lymph nodes, saline treated spleens, cortisol treated spleen and cortisol treated thymus. In the saline treated thymus group, however, the incorporation on the fifth day is significantly greater than it is on the first or fourth days. All statistical significance is at the 95% level or greater in all cases.

Discussion. The spleen, part hematopoietic and lymphopoietic in mice(13,14), did not show as great a decrease in nucleic acid synthesis as the other lymphatic organs. Since the spleen is an organ of mixed cellular population and since cortisol enhances erythropoiesis(13) it is difficult to separate the effects of cortisol on lymphatic elements from its effects on other blood cells. Thus the response to treatment was less marked than it was in the thymus and lymph nodes.

Treatment with cortisol initially produced a decrease in thymidine incorporation. This decrease, however, does not persist with continued treatment. In the thymus and lymph nodes the increases in incorporation of thymidine following 4 and 5 days of cortisol treatment indicate that the remaining cellular population is no longer as sensitive to treatment with cortisol as it was during the first treatment days. Continued treatment with cortisol increased the thymi's ability to inactivate cortisol(10) through biotransformation of the steroid molecule(11,15).

The increased ability of these cells to inactivate cortisol would serve to lessen the effect of this hormone on the synthesis of DNA as is shown by the thymidine incorporation data. The fact that thymidine incorporation increases in the thymus and lymph node after 4 and 5 days of cortisol treatment can be explained on the basis of different cell populations.

The cell population of these organs has been reduced to an immature cell type primarily composed of reticular cells and large lymphocytes(1,18). This immature cell population has the ability to metabolize cortisol to inactive products at a rate greater than normal lymphatic cell populations(10,19). Also cortisol increases the rate of maturation of the daughters of these cells and enhances their subsequent destruction(19). Therefore, even though an increased rate of nucleic acid synthesis occurred after 4 and 5 days of

treatment, we have no net increase in number of cells and, consequently, no increase in organ weights (Fig. 1). It should be noted that the lymph nodes showed the greatest ability to resist the lymphatic tissue effects of cortisol. This is due to the greater percentage of reticular cells present in this organ as compared to the thymus.

The increase in thymidine-2-^{14}C incorporation in the spleen, thymus, and lymph nodes in the saline treated animals (Fig. 2, 3, 4) cannot be fully explained at this time. However, it has been reported that a lymphocytosis in peripheral blood occurs following injection of saline owing to induced stress(16, 17). Injection of either saline or cortisol caused a small decrease in organ weights from the non-injected controls. This decrease can be attributed to the stress produced by the injection in non-adrenalectomized animals.

Summary. The data presented here show that immature cell populations produced by continued treatment with cortisol became resistant to its effects on nucleic acid synthesis after 3 days of treatment in thymus and lymph nodes. The spleen did not show this resistance.

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Extracellular β-Lysin and Muramidase in Body Fluids and Inflammatory Exudates.* (31785)

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β-lysin and muramidase (lysozyme) can be differentiated from each other even though they have similar bactericidal spectra, heat stabilities and adsorption properties(1). Muramidase is present in significant quantities in saliva, tears, plasma, polymorphonuclear cells, and macrophages(2,3). It is absent in aqueous humor, sweat, spinal fluid, and urine. β-lysin is found in both serum and platelets (4). In this study neutralizing anti-β-lysin serum was used in conjunction with conventional assay techniques to identify and quantitate free β-lysin and muramidase in various body fluids and inflammatory exudates of rabbits.

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