Subsequent treatment with uridine reverses the abnormalities of brain, somites and heart by 38%, 28% and 48%, respectively.

Similarly subsequent treatment with thymidine reverses the abnormalities caused by actinomycin D to the extent of 27%, 43% and 33% in brain, somites and heart, respectively. Shortening of axis is also reversed in both the cases.

As the experiments were done uniformly on the same breed, namely white leghorn, inherent variations in the response of individual embryos to actinomycin D are likely to be insignificant. Alleviation with uridine as well as with thymidine appears to be statistically significant. In general overall performance with uridine is slightly better than with thymidine which has been corroborated by appropriate statistical tests, namely test for the significance of proportions, on the assumption of asymptotic normality.

Thus teratogenic effect of actinomycin D in the present system (Figure 1) is seen to be alleviated not only by uridine (Figure 2), a precursor of RNA but also by thymidine (Figure 3) a precursor of DNA indicating that actinomycin D affects DNA biosynthesis as well. Embryos treated with actinomycin D at the head process stage (stage V HAMBURGER and HAMILTON[13]) in which anterior structures are already determined show abnormalities of the posterior axis in that somite formation is very much affected or completely absent while brain, foregut and heart formation though abnormal is not drastically affected (Figure 4). The differential response in the stages No. 4 and 5 towards toxicity of actinomycin D suggests that actinomycin D primarily affects the biosynthesis of the new nucleic acids rather than their functioning. Subsequent treatment with both uridine and thymidine of actinomycin D-treated embryos at the head process stage also show reversal to normal development.

Actinomycin D is known to exert a profound influence on cellular nucleic acids and it interferes with DNA dependent RNA polymerase enzyme (REICH et al.[15], GOLDBERG and RABINOWITZ[16], DENIS[17], BRACHET et al.[1]). Recently it has been suggested by BOVARNICK et al.[18] that actinomycin D affects cell division and possibly DNA replication in *Euglena*. COWELL and WESTON[19] have also shown in avian cell cultures that the uptake of thymidine like that of uridine is suppressed as judged by the pick-up of H^3-TdR in presence of actinomycin D, suggesting inhibition of DNA as well. Teratogenic action of actinomycin D in syrian hamster was found to be reversed to a greater extent with DNA rather than RNA, by ELIS et al.[20]. Our observations are in general accord with the above interpretations as the toxicity of actinomycin D is alleviated by uridine as well as by thymidine. Preliminary studies with guanosine were found to be ineffective in reversing the effect of actinomycin D. It will be of interest to study the effect of actinomycin D by subsequent treatment with deoxy guanosine and cytosine. Further work along this line is in progress.

Zusammenfassung. Untersuchung über die Wirkung von Actinomycin D auf Primitivstreifen- und Entwicklungsstadien der Kopfregion bei Hühnerembryonen in vitro: Anormale Entwicklung des Zentralnervensystems, der Somiten und des Herzens; diese Effekte konnten durch Thymidin oder Uridin zum Teil aufgehoben werden.

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Specific Anti-Antibodies

The concept that antibody globulins show no immunologically recognizable differences from normal globulins has been refuted. Various investigators have demonstrated that anti-antibodies can be produced[1, 2, 6, 7]. The mechanism of their action in blocking specific antibody production is not known; it is believed that anitbodies are directed against the binding site of the antibodies used as antigens[3–5].

Anti-antibodies would be of great significance in a new approach to the treatment of certain allergies and autoimmune diseases and in the prevention of transplant rejection. Induced immunotolerance to white blood cells may pave the way to the treatment of malignant diseases by transfusions or transplantation of immunocompetent cells.

In the past, we have proposed that it might be possible to train individuals' lymphopoietic systems to produce anti-antibodies against anti-bodies in certain immune diseases or in homograft rejection[8]. For this purpose we used an 'immunological triangle' in which 3 animals are involved: a donor and a recipient of the same species and an intermediate animal of a different species. The donor's tissue, red blood cells, was used as antigen to elicit anti-donor antibody in the intermediate species. The anti-donor antibody was then isolated and used as an antigen to elicit anti-antibody in the eventual recipient. This antibody was intended to block antibody formation in the recipient against the donor erythrocytes. This experiment was successful in several animals in which repeated trans-

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fusions of donor red blood cells failed to produce anti-donor antibodies in the recipient[8].

It was believed that more convincing evidence of the existence of anti-antibodies could be acquired by the use of simple antigens such as albumin. This report presents the results obtained by the application of a modification of the immunological triangle concept to the production of anti-antibodies directed at antibodies specific for simple antigens such as albumin, Rh antigen, streptolysin 0 and rheumatoid factor.

Materials and methods[9–11]. Antigens. Commercially available antibodies[12–14] produced in albino rabbits and another species were used to induce the formation of specific anti-antibodies in rabbits. 5 such antibodies were considered appropriate for our purpose: purified anti-human albumin made in horse and rabbit, purified anti-bovine albumin made in goat and rabbit, anti-streptolysin 0 from human sera, and undiluted anti-Rh and rheumatoid factor positive human sera. – Animals. 9 albino rabbits, averaging 2.5 to 5 kg, were used for producing each anti-antibody.

Method. Anti-bovine and anti-human albumin, 5% solution in saline, anti-streptolysin 0, undiluted anti-Rh and rheumatoid factor sera, used as antigens, were mixed with an equal volume of complete Freund's adjuvant for 1 h. $^{2}/_{10}$ of 1 ml of the emulsion thus obtained were injected s.c. in each of 6 different sites selected on the back of the respective animals. On the 21st day following the initial injection, a 10 mg booster dose of the respective antibodies was given i.v. to each animal. A test-bleeding of rabbits was performed on the 28th day of the experiment. If a satisfactory titer of precipitin antibodies was obtained, the animals were bled by cardiac puncture and the sera separated for further studies.

The rabbit anti-antibody sera were tested by immunodiffusion against their respective antibodies and against normal sera of animals in which antibodies were formed. All rabbit anti-antibody sera showed precipitin reactions with both their respective antibodies and with normal sera. To identify the specific anti-antibodies, rabbit anti-antibody sera were absorbed at equivalence with normal sera of the respective animals in which antibodies were produced. Absorbed anti-antibody sera were then tested again against their respective specific antibodies and against normal sera. All but 5 of the absorbed sera showed precipitin reactions with specific antibodies while lacking precipitation with normal sera. The best immunogens were the anti-albumins and the least immunogenic was anti-streptolysin 0.

There was no evidence of anti-antibody formation when rabbit-produced antibodies were used as antigens to induce anti-antibody formation in the same species (rabbits).

Results. Precipitin antibodies against horse anti-human albumin (Figure 1) and against goat antibovine albumin (Figure 2) were demonstrated in sera of all rabbits. These sera also precipitated with normal horse (Figure 1) and goat (Figure 2) sera, respectively. After absorption of rabbit sera with the respective horse and goat normal sera, anti-antibody sera maintained their precipitation power for the specific antibodies (Figure 1 and Figure 2).

Anti-Rh serum produced antibodies in rabbits which precipitated with both anti-Rh (Figure 3) and normal serum of Rh-negative patients (Figure 3). Absorbed rabbit anti-anti-Rh serum with normal human serum from Rh-negative individuals failed to precipitate normal serum but preserved the precipitating power for anti-Rh serum (Figure 3). 2 rabbits in this group failed to produce specific anti-antibodies.

Rheumatoid factor positive serum was used as antigen in 9 rabbits. All animals produced precipitating antibodies against rheumatoid factor positive serum and normal serum. Absorbed rabbit sera with normal serum continued

Agar-gel tests showing precipitin reactions of

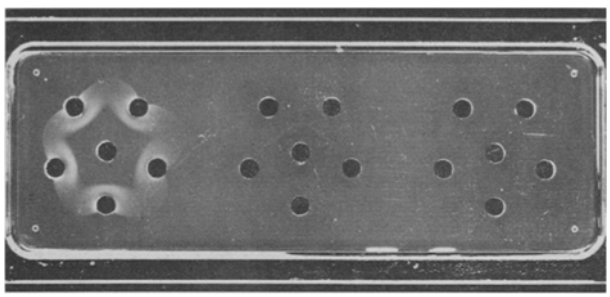

Fig. 1. Human serum albumin with horse anti-human albumin antibody (Pentagone 1); horse anti-human albumin antibody with absorbed rabbit anti-antihuman albumin serum (Pentagone 2) and normal horse serum with absorbed rabbit anti-anti-human albumin serum (Pentagone 3).

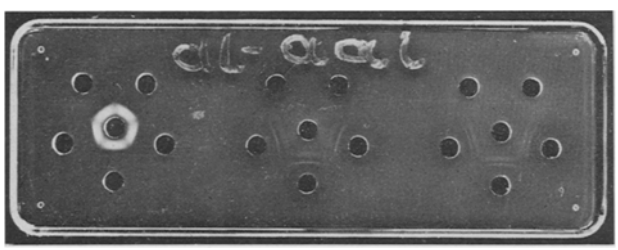

Fig. 2. Bovine albumin with goat anti-bovine albumin anti-body (Pentagone 1); goat anti-bovine albumin antibody (Pentagone 2, lower wells) and goat normal serum (Pentagone 2, upper wells) with rabbit anti-anti-antibovine albumin serum. Goat anti-bovine albumin antibody (lower wells) and goat normal serum (upper wells) with absorbed rabbit anti-anti-bovine albumin serum (Pentagone 3).

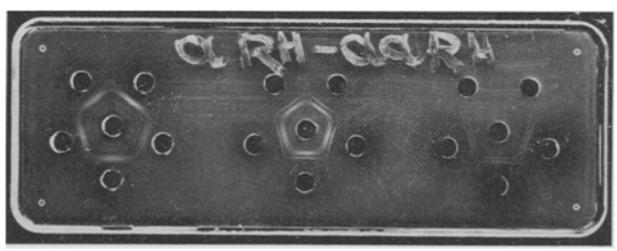

Fig. 3. Human anti-Rh antibody with rabbit anti-anti-Rh serum (Pentagone 1); normal human serum with rabbit anti-anti-Rh serum (Pentagone 2) and human anti-Rh antibody(Pentagone 3, lower wells) and normal human serum (Pentagone 3, upper wells) with absorbed rabbit anti-anti-Rh serum.

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[15] The authors are grateful for the technical assistance of Miss Shirley Schroeder, M.T., A.S.C.P.

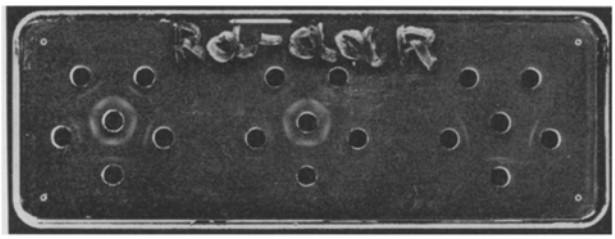

Fig. 4. Rheumatoid factor positive serum and rabbit anti-rheumatoid factor serum (Pentagone 1); normal human serum and rabbit anti-rheumatoid factor serum (Pentagone 2); and rheumatoid factor positive serum (Pentagone 3, lower wells) and normal human serum (Pentagone 3, upper wells) with absorbed rabbit anti-rheumatoid factor serum.

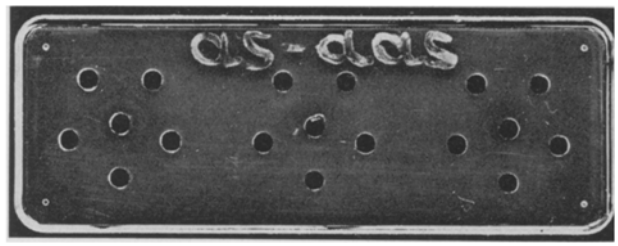

Fig. 5. Human anti-streptolysin 0 antibody with rabbit anti-anti-streptolysin serum (Pentagone 1); human anti-streptolysin 0 antibody (Pentagone 2, 3, lower wells) and normal human serum (Pentagone 2, 3, upper wells) with absorbed rabbit anti-anti-streptolysin 0 serum.

to precipitate the rheumatoid factor positive serum but failed to do so with normal serum (Figure 4).

Only 6 of the 9 rabbits injected with anti-streptolysin 0 produced precipitin antibodies. Their specificity was demonstrated after absorption of rabbit sera with normal human serum (Figure 5).

Discussion. This second report on the production of anti-antibodies by the 'immunological triangle' was intended to demonstrate the presence of specific anti-antibodies to relatively simple antigens such as albumin, streptolysin 0, Rh antigen and rheumatoid factor and confirms our previous work[8].

The results obtained indicate that we might be able to train the lymphopoietic system to produce specific anti-antibodies.

Résumé. Les anti-anticorps pourraient avoir des applications thérapeutiques dans certains états allergiques, les maladies d'auto-immunisation et dans les greffes des tissus. Les auteurs montrent la possibilité de stimuler la formation des anti-anticorps specifiques contre les anticorps produits par des antigènes relativement simples comme l'albumine, le facteur Rh, le facteur rheumatoïde et la streptolysine 0.

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The Growth of Tumour Allografts as a Measure of the Immunosuppressive Potency of Antilymphocyte Sera

The effects of antilymphocyte serum (ALS) on the immune response have been studied extensively and several methods for assessing the potency of different batches have been devised. There is however a need to evaluate all of the methods which may be utilised to assess the immunosuppressive potency of ALS in order to facilitate the development of satisfactory assay techniques which require minimal technical expertise and laboratory facilities.

The ability of ALS to prolong the survival of skin allografts has been exploited in methods for assaying the immunosuppressive potency of samples in vivo[1-3]. Alternative in vivo methods have utilised suppression of the production of plaque forming cells in response to sheep red blood cells[4,5], variations in the distribution of Cr^{51} labelled thymocytes between the liver and the spleen[5] and suppression of xenogeneic graft-versus-host reaction[6].

Initially attempts to assay ALS using in vitro methods were unsuccessful[7,8]. Recently however rosette inhibition[9], opsonization titre[10] and lymphocytophilic antibody titre[11] have been shown to correlate well with the immunosuppressive potency of ALS.

Tumour rejection is inhibited by ALS therapy[12-14] and the ability of ALS to facilitate tumour growth has been utilised to differentiate between active and inactive sera[15-19]. The possibility of exploiting measured changes in the growth rates of tumour allografts to assay the immunosuppressive potency of antilymphocyte sera has not however been explored.

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