

Fig. 2. Participation quantitative de la β -glycoprotéine de la rate, du poumon et de la tumeur au développement de la tumeur de Walker.

Dans le cas de la tumeur au 2-AAF, par exemple, comme l'illustre la Figure 1B, la concentration en β -glycoprotéine n'est augmentée que dans le poumon et seulement à la phase terminale du développement de cette tumeur.

Dans la greffe de la tumeur Walker, par ailleurs, il semble y avoir un cycle de fluctuation de la concentration de cette protéine dans la rate et le poumon, caractérisé par un maximum d'augmentation de concentration dans ces organes au 7^e jour, alors que, dans la tumeur même, l'augmentation relative de cette protéine est progressive tout au cours du développement de cette greffe (Figure 2).

Discussion. Il semble donc y avoir une corrélation entre la concentration tissulaire en cette protéine et le degré d'activité du système réticulo-endothélial. Par exemple, on ne retrouve cette protéine qu'en trace et dans la moëlle seulement, chez le rat exempt de germe; on sait que chez ces animaux, la population cellulaire du système réticulo-endothélial est beaucoup moins importante.

Il serait aussi intéressant, étant donné le rôle de participation de cette protéine au mécanisme de défense², d'établir un lien entre la différence de concentration tissulaire entre le mâle et la femelle et la différence de résistance entre les 2 sexes, vis-à-vis l'agression⁷.

La participation quantitative de cette protéine à la carcinogénèse semble varier suivant la nature de la tumeur induite; cette variation signifie peut-être qu'il existe un rapport direct entre le degré d'agression produit par chaque type de tumeur et l'importance de la réponse du système réticulo-endothélial. Si cette hypothèse s'avérait exacte, il serait alors possible de mesurer à travers cette protéine l'intensité de perturbation métabolique d'un animal par des agents physiques et chimiques variés.

Summary. We show by quantitative radial diffusion in agarose that a β -glycoprotein of tumoral origin present in the lungs, spleen, bone marrow and tumors is present in variable concentrations according to the sex, environment and cycle of experimental tumor development.

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Thymus and Adoptive Transfer of Antibody Formation

Adoptive transfer¹ of antibody production in poultry has been achieved by spleen cells from immune donors²⁻⁴. The present experiment was principally designed to test the capacity of the thymus and bursa of Fabricius to transfer antibody formation in chicken embryos and young chickens.

Adult female New Hampshire chickens hyperimmunized by i.v. injections of human O erythrocytes were used as donors. On the day of sacrifice they showed 10-12 titers (\log_2) of anti-O agglutinins. The thymus, bursa, spleen and muscle were removed aseptically, cut into pieces varying in size from 2 × 3 to 2 × 4 mm, and washed in a kanamycin solution (100 mg/100 ml of Hanks solution). 1 piece of tissue was then placed on the chorio-allantoic membrane⁵ of 12-day-old New Hampshire embryos. An additional group of embryos was grafted with 'dead thymus' from immunized chickens; the thymus cells were killed by repeated freezings and thawings of the

tissue. A number of grafted embryos were sacrificed on the sixteenth, eighteenth and twentieth day of incubation. Young chickens hatched from grafted eggs were bled by cardiac puncture on the first, seventh and fourteenth day. Chickens reared from sham-grafted eggs, and chickens grafted in ovo with the thymus, bursa, spleen, muscle and 'dead thymus' from adult non-immunized chickens served as controls. Birds of all groups (Table) were sacrificed when 15 days old or earlier, and the thymus, bursa,

¹ R. E. BILLINGHAM, L. BRENT and P. B. MEDAWAR, Proc. R. Soc., Ser. B., 143, 58 (1954).

² T. N. MITCHISON, Folia Biol. Praha 3, 72 (1957).

³ Z. TRNKA, Nature 187, 55 (1958).

⁴ L. R. SIBAL and V. H. OLSON, Proc. Soc. exp. Biol. Med. 97, 575 (1958).

⁵ D. L. BALLANTYNE, Transplant Bull. 6, 110 (1959).

spleen, caecal tonsil and several parts of the intestine were fixed in Carnoy and processed for histology. A micro-agglutination technique using 7 × 45 mm tubes, 0.025 ml of a 1% suspension of human O red blood cells and equal volume of serial serum dilutions was used. The peak hemagglutinin titer was determined by microscopic examination of each tube content following a 2-h incubation at room temperature.

Since previous testing of embryonic sera has shown that the actual elaboration of antibody by transferred cells starts in a very small number of embryos at the end of incubation, i.e. 7–9 days after grafting, only the results related to the first, seventh and fourteenth day of post-embryonic life are given in the Table. These results clearly demonstrate that the thymus and spleen are capable of transferring specific antibody synthesis when implanted on the chorioallantoic membrane of chicken embryos, the spleen being more effective than the thymus. On the other hand, the transfer of antibody production cannot be accomplished either by the bursa, muscle and dead thymus from immune donors, or by the thymus, spleen, bursa, muscle and dead thymus from non-immunized birds. The failure of bursal graft from immune donors to confer adoptive immunity gives further support to the view that the bursal cells are not directly engaged in the production of antibody⁶⁻⁸.

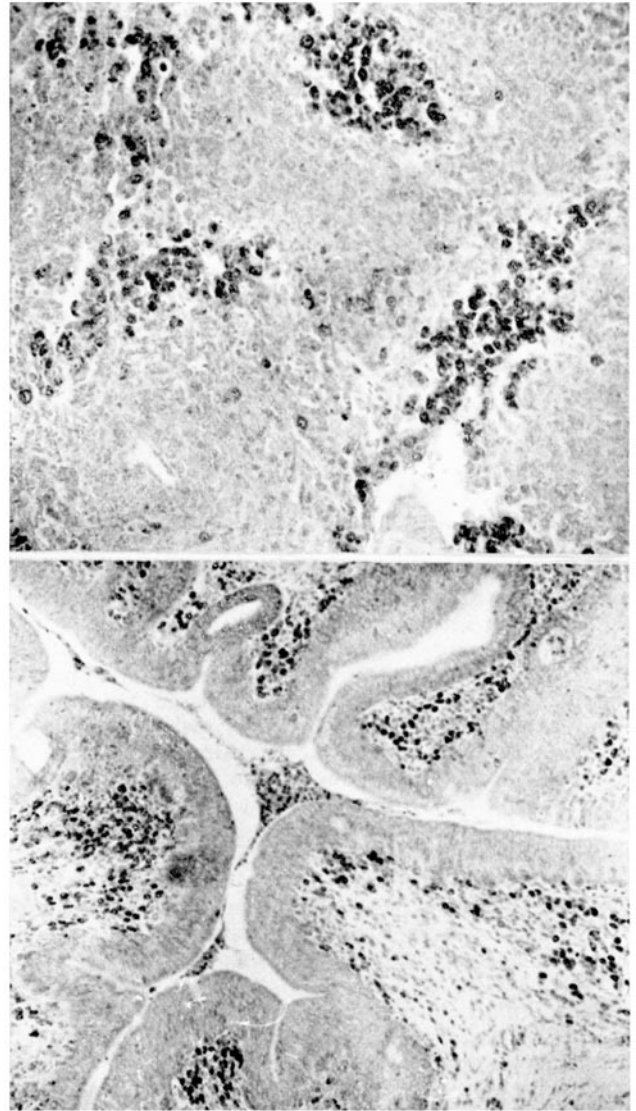
Histological examination revealed the presence of pyronine-positive cells and the formation of germinal centres in the spleen, caecal tonsil and gut of chickens reared from eggs grafted with immune spleen and thymus (Figure). These areas of plasmacellular elements suggest that antibody-producing cells arise in large part from the graft, since similar aggregates of cells were not observed in chickens hatched from eggs which were implanted with muscle and dead thymus from immunized, and with lymphoid and non-lymphoid tissues from unchallenged donors. As we reported earlier, in embryos grafted with thymus, the spleen sinuses are filled with mononuclear cells, many having typical morphology of thymic lymphocytes⁹. Accordingly, the cells of the thymus graft, as well as those of the spleen graft, enter the circulation of the recipient embryo and most probably colonize various lymphoid and non-lymphoid organs.

Anti-O hemagglutinins in chickens hatched from eggs grafted with spleen, thymus, dead thymus, bursa and muscle from chickens hyperimmunized with human O red blood cells

Group	Mean peak antibody titer (log ₂)		
	Age of chickens hatched from grafted eggs (days)		
	1	7	14
Sham-grafted in ovo	0 (0/18)	0 (0/17)	0 (0/15)
Grafted in ovo with			
Spleen	0.7 (4/16) <i>12</i>	3.6 (12/12) <i>11</i>	5.8 (11/11) <i>9</i>
Thymus	0 (0/18) <i>16</i>	2.8 (14/16) <i>14</i>	5.3 (10/10) <i>13</i>
Dead thymus	0 (0/14) <i>12</i>	0 (0/10) <i>11</i>	0 (0/10) <i>10</i>
Bursa	0.1 (2/18) <i>15</i>	0.1 (2/17) <i>12</i>	0 (0/12) <i>10</i>
Muscle	0 (0/17) <i>17</i>	0 (0/15) <i>16</i>	0 (0/13) <i>14</i>

In parentheses: nominator, No. of chickens with hemagglutinins; denominator, No. of chickens in group. Numbers in italics represent No. of chickens grafted in ovo with spleen, thymus, dead thymus, bursa and muscle from adult chickens not immunized with human O erythrocytes. None of these birds demonstrated anti-O agglutinins.

The simplest interpretation of these results is that the thymus and spleen cells from hyperimmunized birds may develop into antibody-forming cells in an allogeneic environment which is immunologically poorly reactive. Because of their previous experience with antigen, a number of the thymus and spleen cells should be considered as carriers of immunological memory. Although it was stated that the memory cells are 'fixed' in the lymphoid organs¹⁰, nevertheless they migrate outward from solid thymus and spleen grafts in order to reach the lymphoid points of the immunologically inexperienced recipient. Apparently,



Top, groups of pyronin-positive cells in the spleen of a 7-day-old chicken grafted in ovo with the thymus from immunized donor; bottom, pyronin-positive cells in the colon of a 7-day-old chicken grafted in ovo with immune spleen (methyl-green-pyronin).

⁶ B. D. JANKOVIĆ and K. MITROVIĆ, *Folia Biol., Praha* 13, 406 (1967).
⁷ P. B. DENT and R. A. GOOD, *Nature* 207, 491 (1965).
⁸ B. GLICK and S. WHATLEY, *Poult. Sci.* 46, 1587 (1967).
⁹ B. D. JANKOVIĆ and K. ISAKOVIĆ, *Nature* 211, 93 (1966).
¹⁰ D. D. MCGREGOR and J. L. GOWANS, *J. exp. Med.* 117, 303 (1963).

these cells survive for at least 23 days (from the twelfth day of incubation to the fourteenth day of postembryonic life). The present report does not offer evidence for the activity of the recipient's own lymphoid cells in the elaboration of anti-O agglutinins.

It is quite possible that the thymus cells are potentially capable of producing antibodies, and the results presented here support the idea that this organ interferes directly with immune reactions. Earlier studies from this laboratory demonstrated that the chicken thymus participates in the development of experimental allergic thyroiditis and parathyroiditis¹¹, contains plasma cells and germinal centres¹², induced a state of immunological tolerance to skin homograft^{9,13}, and shows specifically fluorescing antibody-producing cells following hyperimmunization with a soluble antigen⁶. We also described the ability of the thymus to induce a graft-versus-host reaction when implanted on the ectodermal layer of the chorioallantoic membrane of 12-day-old embryos^{9,13}, a finding which was recently confirmed by GOOD and his associates¹⁴. These facts, in conjunction with the present findings, may provide a solid basis for the belief that the thymus cells are directly involved in the realization of immune responses. It seems reasonable to assume that the thymus plays at least an auxiliary antibody-synthesizing role which may become more pronounced under certain experimental circumstances¹⁵.

Zusammenfassung. Bei Thymus- und Milzimplantation auf die chorioalantoide Membran von Kükenembryonen, die mit humanen O-Erythrozyten immunisiert wurden, wird O-Hämagglutinin erzeugt. Die Bursa ist nicht in der Lage, adoptive Antikörper zu bilden.

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¹¹ B. D. JANKOVIĆ, M. IŠVANESKI, Lj. POPESKOVIĆ and K. MITROVIĆ, *Int. Archs Allergy appl. Immun.* 26, 188 (1965).

¹² K. ISAKOVIĆ and B. D. JANKOVIĆ, in *Germinal Centers in Immune Responses* (Ed. H. COTTIER, N. ODARTCHENKO, R. SCHINDLER and C. C. CONGDON; Springer, Berlin 1967), p. 379.

¹³ K. ISAKOVIĆ and B. D. JANKOVIĆ, *Rev. roum. Biol., Ser. Zool.* 11, 255 (1966).

¹⁴ W. A. CAIN, M. D. COOPER and R. A. GOOD, *Nature* 217, 87 (1968).

¹⁵ This work was supported by grants from the Federal Scientific Fund, Belgrade.

The Adaptive Significance of Blood Potassium and Hemoglobin Types in Sheep

EVANS¹ classified sheep into 2 types on the basis of the concentration of potassium in whole blood; the type which had a concentration of potassium of approximately 35m-equiv/l blood was designated HK and the other type which had a relatively low concentration of potassium (approximately 13m-equiv/l blood) was designated LK. Sheep have also been classified into 3 types, namely A, B and AB, based on the electrophoretic mobilities of their hemoglobins^{2,3}. Both these characters are inherited as simple mendelian alleles^{3,4}. An association between hemoglobin A (Hb A) and HK type has been suggested⁵. In southern Britain the Hb B gene is more common in lowland breeds whereas the Hb A gene predominates in mountain and hill breeds. Hemoglobin A has been found to be more common in northern Europe and northern Britain and relatively uncommon in North Africa, the Middle East and in India⁶.

There are a few reports dealing with potassium types and their association with some physiological characters in Indian sheep⁷⁻¹¹. GHOSH, EYAL and EVANS¹² have studied both hemoglobin types and potassium types in some indigenous breeds of Rajasthan. The report by these workers appears to be the only one in the literature dealing with both these physiological characters – which are thought to have an adaptive significance⁶ – in Indian sheep. The present study was undertaken to determine (1) the gene frequencies for hemoglobin and potassium types in 2 breeds of Indian sheep (Bikaneri and Mandia) and in crossbred sheep (Corriedale × Bikaneri) and (2) to discover whether there was any association between hemoglobin types and either of the potassium types.

Adult Bikaneri (52), Mandia (30) and Crossbred (34) ewes were used (numbers indicate size of respective flocks). They were maintained at Mathura which is situated close

to the arid state of Rajasthan in India. Potassium concentration in the blood was determined using a Beckman spectrophotometer, and hemoglobin type by paper electrophoresis in a Carl Zeiss Jena apparatus.

It is evident from the Table that although the 3 breeds under investigation differed in the gene frequencies for hemoglobin and potassium types, the Hb B and HK genes predominated in all the 3 breeds. The Mandia breed had the highest gene frequency for Hb B and HK. Regression analysis using an IBM 1620 computer showed no significant correlation between gene frequencies for Hb B and HK (data used were from the present work and that of GHOSH et al.¹²). However, it is seen from the Table that in all 3 flocks, the frequencies for the Hb B and HK genes were both quite high. These findings are contrary to those of EVANS et al.⁶ who found that there was a correlation

¹ J. V. EVANS, *Nature* 174, 931 (1954).

² H. HARRIS and F. L. WARREN, *Biochem. J.* 60, 29 (1955).

³ J. V. EVANS, J. W. B. KING, B. L. COHEN, H. HARRIS and F. L. WARREN, *Nature* 178, 849 (1956).

⁴ J. V. EVANS and J. W. B. KING, *Nature* 176, 171 (1955).

⁵ J. V. EVANS, H. HARRIS and F. L. WARREN, *Proc. R. Soc. B* 148, 249 (1958).

⁶ J. V. EVANS, XIth Congr. Int. Soc. Hemat., Sydney (1966).

⁷ G. C. TANEJA and P. K. GHOSH, *Indian J. exp. Biol.* 3, 166 (1965).

⁸ G. C. TANEJA, B. M. FULADI and R. K. ABINCHANDANI, *Indian J. exp. Biol.* 4, 125 (1966).

⁹ G. C. TANEJA and R. K. ABINCHANDANI, *Indian J. exp. Biol.* 5, 226 (1967).

¹⁰ G. C. TANEJA, *Experientia* 23, 645 (1967).

¹¹ G. C. TANEJA and P. K. GHOSH, *Indian Vet. J.* 44, 402 (1967).

¹² P. K. GHOSH, E. EYAL and J. V. EVANS, *Aust. Arid Zone Res. Conf., Alice Springs, N.T.* (1965).