ing with a bis(2-amino-1-naphthyl) phosphate. In the first stage, one mole of 2-amino-1-naphthol is released plus mono-aryl phosphate and in the second, the mono-aryl phosphate is hydrolyzed.

Phosphate conjugates have not been described previously as end products of metabolism of foreign compounds. Three features make this compound a promising candidate for the active bladder carcinogen: 1. The compound is readily split by phosphatases occurring in the dog urine to form 2-amino-1-naphthol which has been shown to be an active carcinogen(7). 2. The compound has solubility properties characteristic of compounds capable of crossing the cell wall. 3. Preliminary observations in rabbits injected with 2-naphthylamine indicate the absence of similar phosphate conjugates. Boyland and Manson(8) report the absence of 2-amino-1-naphthyl phosphate in the rat. Both these species are relatively insensitive to bladder cancer when exposed to 2-naphthylamine, while the dog, which elaborates this phosphate conjugate, is sensitive to the disease.

*Summary.* Bis(2-amino-1-naphthyl) phosphate has been demonstrated as a urinary end product of 2-naphthylamine metabolism. Phosphate conjugates have not been observed previously as urinary end products. The compound may be related to the occurrence of bladder cancer in this species upon ingestion of 2-naphthylamine.

The contribution of Miss Joan Royce in this work is gratefully acknowledged.

1. Troll, W., Nelson, N., *AIHA Journal,* Dec. 1958.
2. Troll, W., Belman, S., Nelson, N., Levitz, M., Twombly, G. H., PROC. SOC. EXP. BIOL. AND MED.,
3. Clayson, D. B., *Biochem. J.,* 1950, v47, xxii.
4. Boyland, E., Manson, D., Sims, D., Williams, D. C., *ibid.,* 1956, v62, 68.
5. Boyland, E., Gasson, J. E., Williams, D. C., *Brit. J. Cancer,* 1957, v11, 120.
6. Lowry, O. H., Lopez, J. A., *J. Biol. Chem.,* 1946, v162, 421.
7. Clayson, D. B., Jull, J. W., Bonser, G. M., *Brit. J. Cancer,* 1958, v12, 222.
8. Boyland, E., Manson, D., *Biochem. J.,* 1955, v60, ii.

Received October 27, 1958. P.S.E.B.M., 1959, v100.

## Ocular Transplants of Joint as an Experimental Method.* (24545)

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An experimental polyarthritis can be produced in rats and mice using species-specific strains of *Micoplasma arthritidis* (pleuropneumonia-like organism) in broth culture administered either intravenously or intraperitoneally. This experimental infection in rodents has served as a method for evaluating the effect of various environmental conditions and chemotherapeutic agents(1). It has been possible to segregate drugs into 3 groups: 1) those which prevent or cure the arthritis; 2) those which worsen its course; and 3) those without apparent effect. Because of the tendency for joint involvement to characterize this experimental infection, we wondered whether joint involvement would also appear in transplanted joint tissues. To approach this problem we adapted a technic previously employed for transplanting tumor(2) and ovarian tissues(3) to the anterior chamber of the rat eye. The first experiment was an effort to explore the possibility of intra-species (homologous) transplantation of rat joint tissues to the anterior chamber and to determine the possibility of "arthritic" changes in the transplanted tissue following intraperitoneal infection with broth cultures of *Micoplasma arthritidis* (pleuropneumonia-like organism). If "arthritic" changes could be produced in the

* This investigation was supported, in part, by research grant A-1346 from Natl. Inst. of Arthritis and Metabolic Diseases.

TABLE I. Homologous Transplantation of Joint Tissues to the Anterior Chamber.

| Exp. | Donor | Tissue | Recipient | No. of rats* | Results |
|---|---|---|---|---|---|
| 1 | 17-day-old embryo | knee joint | mature‡ | 5 | 5/5 takes. Vascularized, grew to fill chamber by 11 days after transplantation. |
| 2 | newborn | *Idem* | " | 10 | 10/10 takes. Vascularized, grew to fill ⅔ of chamber by 39 days after transplantation. |
| 3 | immature† | synovia and cartilage from knee | immature | 7 | 7/7 takes. Vascularized, no growth. Sacrificed 21 days after transplantation. |
| 4 | mature | *Idem* | mature | 6 | 6/6 no takes. Only few fine vessels which degenerated 10 days after transplantation. Tissue became fibrous. |

* Transplants were made to both eyes. † 45-day-old. ‡ 120-day-old.

transplanted tissue, such changes could be readily observed through the cornea and might thus serve to indicate the effect of various drugs (Table I). In the second experiment, the recipient rats with ocular transplants of joint tissues were infected and medicated with various drugs for chemotherapeutic study (Table II).

*Materials and method.* Transplantation was done with entire knee joints minus the skin from both 17-day-old embryos and newborn rats. The donor animal was sacrificed, the knee joint excised, skinned, and placed in physiological saline. Synovial tissue with adhering cartilage from knee joints was also taken from immature, young adult and mature rats for transplantation. The recipient animal was anesthetized with ether and the tissue was transplanted by Goodman's technic (4) into both eyes. Tissues taken from male

TABLE II. Joint Transplantation for Chemotherapeutic Study.

| Group | No. of animals | Donor | Tissue | Recipient | Treatment | Results |
|---|---|---|---|---|---|---|
| I | 12 | immature* | synovia and cartilage from knee | immature | 3 controls | NCT§ |
| | | | | | 3 infected (3 days after transplantation). | ⅔ rat arthritic. NCT |
| | | | | | 3 infected (3 days after transplantation) and medicated with 0.5% phenylbutazone in ground food (Butazolidin®). | ⅔ rat arthritic. NCT |
| | | | | | 3 uninfected and medicated with 0.5% phenylbutazone in ground food. | NCT |
| II | 10 | " | *Idem* | young adult† | 3 controls | NCT |
| | | | | | 3 infected (46 days after transplantation). | |
| III | 12 | " | " | mature‡ | 2 control | NCT |
| | | | | | 2 infected (2 days after transplantation) and treated with gold thioglucose in sesame oil (Solganol B®) 1 day prior to transplantation. | ½ rat arthritic. NCT |
| | | | | | 2 infected (2 days after transplantation). | ½ rat arthritic. NCT |
| IV | 9 | young adult | " | young adult | 3 controls | NCT |
| | | | | | 3 broth controls | NCT |
| | | | | | 3 infected (8 days after transplantation). | ⅓ rat arthritic. NCT |

* 45-day-old. † 90-day-old. ‡ 120-day-old. § No change in transplant.

donors were transplanted to male recipients and tissues taken from female donors were transplanted to female recipients. All rats used in our experiments were of the Wistar strain.

*Results.* Observations were made through the cornea every 3 days and histologic preparations were made of the transplants excised at various times after insertion into the anterior chamber. The tissues were immediately placed in 10% formalin after excision and stained with Meyer's haematoxylin and eosin. Criterion of viability ("takes") was based on growth and differentiation, vascularization, and maintenance for 15 days or longer without becoming fibrous or resorbed. All transplants that did not "take" due to technical error have been omitted from the data.

The results as given in Table I indicate that intraspecies (homologous) transplants were successful with the best results obtained with embryonic and newborn tissues, which grew rapidly to fill the chamber. The older tissues tend toward fibrosis and degeneration. Immature tissues failed to grow, but became vascularized and did not become fibrotic during the various experiments.

In spite of good development of polyarticular arthritis in rats infected intraperitoneally with broth cultures of *Micoplasma arthritidis* (pleuropneumonia-like organism), the tissues transplanted to the anterior chamber of these infected animals did not show gross changes characteristic of the rat polyarthritis. The use of various medications did not alter the situation, although in previous experiments many drugs have either worsened, prevented, cured, or have not changed the course of the rat disorder (Table II). The histologic sections did not enable us to grade the inflammatory cellular reaction due to infection and due to transplantation. In 2 animals with transplants and infected with *Micoplasma arthritidis* (pleuropneumonia-like organism), we have obtained negative cultures of the anterior chamber fluid.

*Discussion.* Thus far, it would appear that there is a "barrier" in the anterior chamber, supporting Greene(1). Also, the anterior chamber may lack something necessary to cause changes characteristic of the rat polyarthritis. Perhaps the presence of synovial tissue and the *Micoplasma arthritidis* (pleuropneumonia-like organism) may not be enough. There may be a third factor involved in causing rat arthritis. For example, the aqueous in the anterior chamber may not support growth of *Micoplasma arthritidis* (pleuropneumonia-like organism); we may not have kept the tissues in the eye long enough to adjust to the new environment before infection. These questions and problems are presently under study in our laboratory. We have studied in one group of animals the protein pattern by electrophoresis and found the beta globulin elevated in the infected animals.

*Summary.* 1) Intra-species (homologous) rat joint tissue transplants were successful. 2) Production of arthritis by intraperitoneal injection of broth culture of *Micoplasma arthritidis* (pleuropneumonia-like organism) did not produce gross or microscopic evidence of arthritis in the transplanted joint tissues although the recipient animal developed a good polyarticular arthritis. 3) The use of gold thio-glucose and phenylbutazone in limited numbers of infected rats did not alter the above situation. 4) Failure of transplanted joint tissues to become arthritic in infected animals suggests an ocular barrier.

1. Kuzell, W. C., Mankle, E. A., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 677.

2. Greene, H. S. N., *Ann. N. Y. Acad. Sci.*, 1955, v59, 311.

3. Noyes, R. W., Yamate, A. M., Clewe, T. H., *Fertil. and Steril.*, 1958, v9, 99.

4. Goodman, L., *Anat. Rec.*, 1934, v59, 223.

Received October 27, 1958. P.S.E.B.M., 1959, v100.