TABLE I. Efficiencies of Killing by $^{32}P$ Decay.

| Organism | Investigator | $\alpha$ (efficiency) |
|---|---|---|
| øX-174 | Tessman(8) | 1.1 |
| Phage T2 | Stent and Fuerst(9) | .12 |
| *E. coli* | Fuerst and Stent(10) | .02 |
| D98/AG | Ragni and Szybalski(7) | .00023 |
| KB | Leach | .0004* |

Calculated from the equation $\log_{10}s = -1.48 \times 10^{-6}\ \alpha\ A_0N(1-e^{-\alpha t})$ in which s is number of survivors, $A_0$ is specific activity of the medium in mC/mg P, N is number of phosphorus atoms per cell in DNA, and t is number of days. A value of $2 \times 10^{10}$ DNA-P atoms was used in this study.
* Calculated from 0.1 and 0.5 mC/mgP levels.

about every tenth disintegration is lethal and Stent and Fuerst have postulated that to be lethal a decay must occur opposite a naturally occurring break in the double stranded DNA for chain breakage. *E. coli* is more resistant, possibly because it has several nuclei under common growth conditions which could undergo intracellular recombination to maintain viability. A study of the effect of bromodeoxyuridine substitution on mammalian cell radiosensitivity by Ragni and Szybalski(7) reveals that the Detroit 98 strain of cells is also radioresistant. They found only one level of resistance—not a biphasic curve. The possibilities resulting in greater resistance are that only a small portion of the genome must function for a mammalian cell to plate (give rise to a colony) under the tissue culture conditions imposed and that the cells are polyploid. The polyploid nature of the cells makes possible intracellular recombinations to yield the same result as multiplicity reactivations in phages. Similar postulates were made by Ragni and Szybalski(7). As would be expected from the previous data the efficiency of killing of cells labeled with $^{32}P$ activities greater than 1 mC/mg was lower than $4 \times 10^{-4}$. However, their viability decreased with time (decay of $^{32}P$) suggesting that the more resistant cells also contained $^{32}P$.

The lack of sensitivity of mammalian cells cultured *in vitro* to $^{32}P$ decay when incorporated into DNA makes impractical the application of this tool in the elegant way of Stent and co-workers. The existence of cells with 2 levels of sensitivity to $^{32}P$ disintegration in mammalian cell cultures is described.

1. Stent, G. S., Fuerst, C. R., *Advanc. Biol. Med. Phys.*, 1960, v7, 1.
2. Uchida, H., Stent, G. S., *J. Mol. Biol.*, 1960, v2, 251.
3. ———, *ibid.*, 1960, v2, 262.
4. Grosch, D. S., Sullivan, R. L., *Biol. Bull.*, 1953, v105, 296.
5. King, R. C., *J. Exp. Zool.*, 1954, v126, 323.
6. Oftedal, P., Mossige, J., *Nature*, 1957, v179, 104.
7. Ragni, G., Szybalski, W., *J. Mol. Biol.*, 1962, v4, 338.
8. Tessman, I., *Virology*, 1959, v7, 263.
9. Stent, G. S., Fuerst, C. R., *J. Gen. Physiol.*, 1955, v38, 1441.
10. Fuerst, C. R., Stent, G. S., *ibid.*, 1956, v40, 73.

Received October 30, 1963. P.S.E.B.M., 1964, v115.

## Passive Transfer of Human Allergies to Prosimians: Skin-Reactions in the Lemuroid, *Nycticebus coucang* (Slow Loris). (29001)

LAURENCE L. LAYTON AND FRANK C. GREENE
*Western Regional Research Laboratory,* Albany, Calif.*

Layton and coworkers have shown(4,5) that human atopic hypersensitivities of asthma and hay fever can be passively transferred by serum from allergic human beings to nonhuman primates of suborder *Anthropoidea*. Most species of New World monkeys and Old World monkeys, apes, and men exhibit essentially the same capacities to bind atopic reagins of human sera in skin and to exhibit reactions in the passively sensitized sites when challenged with the appropriate atopens (allergens).

Anthropologists generally believe that the

* A laboratory of the Western Utilization Research and Development Division, Agri. Research Service, U.S. Dept. of Agriculture.

Old World *Anthropoidea* (monkeys, apes, and man) and the New World primates evolved, by parallel development, from different lemuroid prosimians. For this reason we were somewhat surprised to find(5) that human atopic reagins will sensitize most of the New World monkeys including the *Hapalidae* (marmosets). Acceptance by nonhuman primates of passive sensitization with human allergic sera might be referred to as "atopic reagin recognition." The occurrence of this phenomenon in New World primates supports the view that the Old World and New World *Anthropoidea* share a blood relationship derived from a common primate or "preprimate" ancestral stem species.

Work by Nuttall(8), by Boyden and coworkers(1), and others(3) suggested that the relationship between Old World monkeys and New World monkeys is more remote than is the relationship between Old World monkeys and man. Precipitin tests showed that there is only a slight serological relationship between the *Anthropoidea* and the *Prosimii* represented by the lemuroids of Madagascar (1,8). Montagna and coworkers(6,7) have made comparative histological studies upon the skins of the major genera of primates of both the *Anthropoidea* and the *Prosimii.* Montagna's studies tended to support the conclusions of Nuttall(8) and of Boyden and coworkers(1) which were based upon precipitin tests with rabbit antisera(1,3,8). It has been suggested(2,6,7,9,10) that the slow loris *(Nycticebus coucang),* and possibly the tree shrews *(Tupaia),* are primitive prosimians and might be considered "living fossils," relatively unchanged since the beginnings of the primate order 60 to 80 million years ago. On the other hand, the *Tupaia* may be *Insectivora* and the contemporary representatives of the remote genetic link between the primates and the *Insectivora,* i.e., not so much a lemuroid as they are a "preprimate" form.

Slow lorises are easily obtained from Asia; hence we decided to determine whether or not the skin of this primitive species would bind human atopic reagins and exhibit passive allergic reactions when challenged with appropriate allergens.

*Experimental.* Materials. A male and a female slow loris were obtained from India. Both of the lorises were in poor physical condition. The male was debilitated by starvation and refused to eat. The female at first ate only a small amount of fruit, but later, after the first tests, ate mice and fruit. The female is shown in Fig. 1A and 1B.

Sera for this study were from 7 French patients allergic to castor beans, and from 4 American patients allergic to ragweed pollen. Both groups of sera had been tested upon several species of monkeys, an ape, and a baboon(5) and shown to contain skin-sensitizing antibodies (atopic reagins). The sera were not diluted and were used to sensitize the animals passively.

The castor bean seed protein (allergen) for this study was taken from the same sample used in the earlier studies of serum transfer to *Anthropoidea*(5). Allergenic extracts of dwarf ragweed pollen were obtained from a commercial source.

*Methods.* The undiluted sera (0.05 ml aliquots) were injected intracutaneously into the abdomen, neck, and limbs of the lorises. Subcutaneous injection of serum will not sensitize the local skin site(5).

Forty-eight hours afterwards the animals were injected into the exposed saphenous vein with 0.5% solution of Evans blue dye in physiological saline at a dosage of 2 ml per kg of body weight. No nonspecific leakage of dye occurred in any of the passively sensitized sites.

The male loris was challenged i.v. with 5 mg of castor bean seed protein in 1 ml of isotonic saline. The reactions in this animal were recorded and photographed. The female was then challenged. Both animals were photographed in color before challenge and again when no further reactions could be detected after a challenge with castor bean. They were then challenged with 0.3 ml of the dwarf ragweed extract.

Two weeks after the first tests the surviving female loris was in an excellent state of nutrition. She was sensitized and tested to check certain unexpected reactions described below.

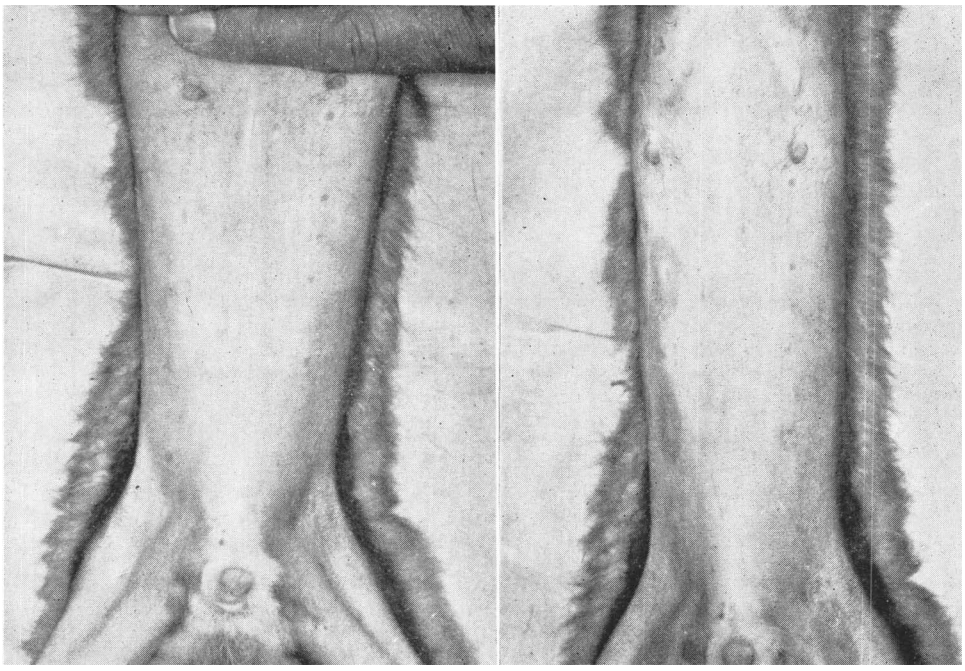

FIG. 1. Passive allergic reactions in skin of the lemuroid, *Nycticebus coucang,* passively sensitized by reaginic sera from human patients. A. Ten min after intravenous injection of Evans blue dye and just prior to challenge with castor bean allergens. (Injection sites were spotted with dilute picric acid for ease of location.) B. Fifteen min after intravenous challenge with castor bean protein. Extravasation of dye is evident in the castor bean sensitive sites on right side of abdomen and on both thighs. Unreacted sites on the left side were sensitized to ragweed pollen and hence served as controls during castor bean challenges.

*Results and discussion.* In the male loris, skin reactions could be detected within about 2 minutes after injection of the castor bean allergen. First, the reacting sites showed mild erythema, followed from 2 to 5 minutes later by wheal formation in the centers of the sensitized sites. The wheals and erythema subsided without any extravasation of dye, and we hastened to photograph the lesions which were disappearing rapidly. The photographs showed no detail beyond residual faint blanching in the reaction sites. Blebs of saline injected into the abdomen also failed to elicit extravasation at sites of irritation.

In the female loris, intravenous challenge with the allergenic extracts elicited similar biphasic reaction. However, the whealing phenomenon was followed in about 15 minutes by extravasation of dye into the reacting sites. This animal was photographed just prior to challenge (Fig. 1A) and again after challenge with castor bean protein (Fig. 1B). In the second series of tests upon the female, made 2 weeks later, extravasation of dye occurred simultaneously with the erythema of whealing, hence the previously observed dissociation of phases could not be detected in the healthy, well-fed animal.

The polyphasic skin reaction observed in both of the lorises when they were in poor physical condition due to starvation contrasted greatly with the immediate extravasation of dye in one of these animals when it was healthy. The immediate and simultaneous piloerection, blanching and extravasation in allergic skin reactions in the well-fed loris were similar to the passive allergic skin reactions observed in monkeys(5). The lesions in the skin of the loris are shown in Fig. 1B.

Since the healthy loris exhibited passive allergic skin reactions which appeared to be similar to those observed in the majority of monkeys, it might be safe to assume that the allergic skin reactions in the *Anthropoidea*

TABLE I. Allergic Serum Transfer Test. Allergic skin reaction in passively sensitized monkeys, and prosimian.

| Patient No. | Allergy | Intensity of passive reaction in test animals: Previous tests upon monkeys | Slow loris |
|---|---|---|---|
| 1 | Castor bean | 4+ | 4+ |
| 2 | " " | 4+ | 3+ |
| 3 | " " | 4+ | 3+ |
| 4 | " " | 4+ | 3+ |
| 5 | " " | 4+ | 4+ |
| 6 | " " | 4+ | 4+ |
| 7 | " " | 4+ | 3+ |
| 8 | Ragweed | 4+ | 3+ |
| 9 | " | 4+ | 1+ |
| 10 | " | 4+ | ± |
| 11 | " | 4+ | ± |

4+ = 20 mm lesion.
3+ = 15 " "
1+ = 5 " "
± = Erythema without extravasation of dye.

are also polyphasic but that the phenomena occur so rapidly that they appear to be nearly simultaneous. In passively sensitized apes and monkeys, the allergic skin reactions usually appear first as piloerection and blanching followed immediately by extravasation of dye. Allergic whealing is rare in monkeys, and when it does occur it usually follows extravasation.

Challenge with ragweed pollen elicited mild reactions in passively sensitized sites on the lorises. Observations are tabulated in Table I.

*Conclusions.* Human reaginic sera that passively sensitized the major species of the suborder *Anthropoidea* also passively sensitized the lemuroid prosimian, *Nycticebus coucang* (slow loris). In the slow loris, severe starvation appeared to cause dissociation of the phenomena accompanying the skin reactions in passively sensitized skin sites. The skin reactions in the healthy, well-fed loris were similar to the corresponding reactions in monkeys and apes *(Anthropoidea).*

Human atopic reagins will not ordinarily confer persisting passive sensitivity to the skin of nonprimates. To the extent that they do confer passive sensitivity, a preponderance of passive allergic skin reactions in atopic reagin-sensitized individuals of a given species may indicate some degree of blood relationship with the genus *Homo.* Failure to exhibit passive sensitization by human atopic reagins may be much less significant, since a small percentage of human volunteers and monkeys do not exhibit passive sensitization with human reaginic sera. Similar transfer studies to *Tupaia* and other orders of mammals are now in progress.

*Summary.* Human serum transfer tests were conducted upon the lemuroid prosimian, *Nycticebus coucang* (slow loris). Intradermal injections of allergic sera passively sensitized the skin of the slow loris. Challenge with the allergens affecting the human patients caused erythema, piloerection, blanching, whealing, and extravasation of intravenously injected colloidal dye in the passively sensitized sites. The human allergic serum transfer test may be an aid in ordinal and subordinal classification of species suspected of being related to the *Anthropoidea.*

Mr. Leonard Harrison, animal caretaker, rendered invaluable aid in maintaining the animals in healthy condition. Mr. Wm. G. Murray filmed the procedures and performed the photographic services. Dr. Raphael Panzani of Marseille, France, supplied the sera from castor bean allergic patients. Dr. C. W. Denko, Ohio State University, supplied the sera from ragweed atopic patients.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Dept. of Agriculture to the exclusion of others that may be suitable.

1. Boyden, A., Gemeroy, D., DeFalco, R., *Serological Museum Bull.,* 1956, No. 16: 3-8, Rutgers Univ., New Brunswick, N. J.

2. Gregory, W. K., McGregor, J. H., *Encycl. Brit.,* 1948, v18, 485-490D, Encyclopedia Britannica, Inc., Chicago.

3. Landsteiner, K., Miller, C. P., *Science,* 1925, v61, 492.

4. Layton, L. L., Lee, S., DeEds, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 623.

5. Layton, L. L., Greer, W. E., Greene, F. C., Yamanaka, E., *Int. Arch. Allergy,* 1963, v23, 87.

6. Montagna, W., Yasuda, K., Ellis, R., *Am. J. Phys. Anthrop.,* 1961, v19, No. 1, 1-22.

7. Montagna, W., Yun, J. S., Silver, A. F., Quevedo, W., *ibid.,* 1962, v20, No. 4, 431.

8. Nuttall, G. H. F., *Blood Immunity and blood relationship,* Cambridge Univ. Press, 1904, 161.

9. Simpson, G. G., *The principles of classification*

*and a classification of mammals, Bull. Am. Museum Nat. Hist.*, 1945, v85, 181.

10. ———, *The meaning of evolution*, Yale Univ. Press, New Haven, 1949, p364.

Received October 30, 1963. P.S.E.B.M., 1964, v115.

## Carbohydrate Metabolism in *Pasteurella multocida*. III. Incorporation of Glucose Carbon into the Cell-Wall Polysaccharide.* (29002)

INDA S. M. DE ISSALY† AND A. O. M. STOPPANI

*Institute of Biochemistry, School of Medicine, University of Buenos Aires, and Laboratory for Cell Metabolism, National Atomic Energy Commission, Argentina*

A partially purified lipopolysaccharide antigen isolated by phenol extraction from *Past. septica (multocida)* was found to contain glucosamine, galactose and aldoheptose(1). This communication deals with the complete purification of the polysaccharide moiety of the antigen and its relationship to glucose metabolism.

*Materials and methods* were the same as used previously(2,3). Glucose-U-$C^{14}$ (0.15 mC/mmole) was from the Radiochemical Centre, Amersham, Great Britain. Hexosamines were revealed in the chromatograms with the Elson-Morgan reaction(4). Paper electrophoresis was performed as described by Markham and Smith(5).

*Results and discussion. Purification of polysaccharide. Step 1* (removal of capsular polysaccharide(6)). 9 g of acetone-dried *Past. multocida* (strain Beufort No. 28 from the Institute Pasteur, Paris) were shaken mechanically with 150 ml of 2.5% NaCl and 1 ml of toluene for 4 hours at 20°. The suspension was centrifuged, the supernatant poured off and the residue reextracted twice as above. The cells were suspended in 100 ml of boiling distilled water and shaken gently with glass beads for 2 minutes. After centrifugation at 12000 *g* the extraction was repeated with 50 ml of distilled water at 100° and the centrifugation was repeated. *Step 2* (extraction). The cell residue was suspended in 90 ml distilled water, warmed at 65° and treated with an equal volume of aqueous 90% (w/v) phenol solution at 65°(7,8). The mixture was stirred for 45 minutes at this temperature and then cooled rapidly at —2°. Upon centrifugation, the phenol phase was discarded. *Step 3* (acetone precipitation). The aqueous solution was poured into 10 vol of dry acetone at —10°, left for 24 hours at —20° and centrifuged. The sediment was washed 3 times with acetone at —10°, suspended in 20 ml distilled water, dialysed for 48 hours against running tap water and dried under vacuo. Yield: 708 mg. *Step 4* (removal of nucleic acid). An aqueous 1% (w/v) solution of polysaccharide was centrifuged twice at 100000 *g* for 3 hours. The sediment was dissolved in 60 ml distilled water and treated twice as above with cold acetone containing 0.3% of potassium acetate. The residue was dried under vacuo, redissolved in distilled water and centrifuged once at 100000 *g* for 3 hours. The pellet was dissolved in 10 ml distilled water, dialysed against distilled water for 48 hours at 4° and dried under vacuo. White colorless leaflets were obtained. Yield: 204 mg. Nucleic acid and protein content (determined spectrophotometrically(9)) were 0.2 and 0.6% respectively. *Step 5* (removal of lipid(10)). The polysaccharide was dissolved in 25 ml 0.01 N HCl at 100° for 45 minutes. The solution was cooled, centrifuged at 3000 rpm for 30 minutes, the pellet washed twice in the centrifuge with 0.01 N HCl and dried under vacuo. Yield: 134 mg. *Step 6* (ethanol precipitation). The polysaccharide was dissolved in

* This investigation was supported by grants from The Rockefeller Foundation and Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

† Inda M. S. de Issaly was on leave from Inst. Nac. de Microbiol.