24 h) and 0·10–0·50 mg nicotine (in 24 h) under normal conditions, 0·30–0·90 mg cotinine (in 22 h) and 0·50–0·80 mg nicotine (in 8 h) when the urine was made acidic after the oral administration of ammonium chloride, and 0·30–0·40 mg cotinine (in 36 h), and less than 0·06 mg nicotine (in 36 h) when the urine was made alkaline after the oral administration of sodium bicarbonate.

Absorption of nicotine from the lungs and excretion in the urine were found to be extremely rapid, as the greatest rate of excretion was in the period 0–15 min after smoking. The rate of nicotine excretion under acidic conditions fell very rapidly to less than 0·1 μg/min 8 h after smoking the two cigarettes. The elimination of nicotine did not appear to follow first order kinetics, and this is being further investigated. The peak level of cotinine excretion was found to be at about 2 h after smoking.

The subjects were heavy smokers (twenty cigarettes a day), and intermittent smokers (between two and five cigarettes a week), and for the purpose of the experiments inhaled the smoke from the non-filter cigarettes used.

We thank Prof. H. McKennis, jun., for generously supplying us with cotinine and the Tobacco Research Council for financial assistance.

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[1] Jakzkewitz, H., *Hoppe-Seyler's Z. Physiol. Chem.*, **292**, 94 (1953).
[2] Fabre, R., *et al.*, *Ann. Pharm. Franc.*, **10**, 579 (1952).
[3] Desoille, H., *et al.*, *Arch. Mal. Prof.*, **24**, 422 (1963).
[4] Ganz, A., and Kelsey, F. E., *Fed. Proc.*, **9**, 274 (1950).
[5] Quin, L. D., and Pappas, N. A., *Agric. Food Chem.*, **10**, 79 (1962).
[6] Goldbaum, L. R., and Domanski, J. J., *Prog. in Chem. Toxicol.*, **2**, 221 (Academic Press Inc., New York, 1965).
[7] Beckett, A. H., Rowland, M., and Triggs, E. J., *Nature*, **207**, 200 (1965).
[8] McNiven, N. L., *et al.*, *Nature*, **208**, 788 (1965).
[9] Haag, H. B., and Larson, P. S., *J. Pharm. Exp. Therap.*, **76**, 235 (1942).

HAEMATOLOGY

Rapid Induction of *in vivo* Fibrinolysis with D-Gluconyl-L-tyrosine

RECENTLY I reported[1] lysis of thrombi in the veins and arteries of animals with sodium gluconate and a dialysable constituent of fibrin extract or whole blood. The ultraviolet absorption spectrum of fibrin extract displayed a maximum at 275 mμ and a minimum at 247 mμ. These values happen to coincide closely with those recorded for tyrosine. This finding suggested that L-tyrosine might be successfully substituted for fibrin extract and, if this was so, that a peptide formed from the amino-acid and gluconic acid would also be fibrinolytic.

Clots were induced in the jugular veins of dogs as previously described[1], and infusions administered locally without the admission of blood. A mixture of 100 mg of L-tyrosine and 1 g of sodium gluconate lysed such thrombi, while L-alanine with gluconate or L-tyrosine with sodium glucuronate were inactive. D-Gluconyl-L-tyrosine was prepared by the method of Iacobellis[2]. One hundred milligrams in 1 ml. of distilled water injected into a leg vein was sufficient to restore within 15 min the flow of blood in occluded jugular veins of dogs weighing 12–13 kg.

The compound did not lyse fibrin clots *in vitro*. It may be visualized as the prosthetic group of a larger molecule, presumably a protein.

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[1] Kopper, P. H., *Nature*, **211**, 43 (1966).
[2] Iacobellis, M., *Arch. Biochem. Biophys.*, **59**, 199 (1955).

IMMUNOLOGY

Complement-fixing Antibodies in Monkeys bearing Tumours induced by Rous Sarcoma Virus

ROUS sarcoma virus (RSV) produces tumours in rodents, monkeys, sheep and dogs[1–7]. RSV tumours in hamsters, guinea-pigs and rats contain complement-fixing (CF) antigens which elicit antibodies to homologous virus-free RSV-induced hamster tumour antigens, virus-containing RSV-induced chicken tumours, and antigens induced by the growth of several strains of avian leukosis viruses in chicken embryo fibroblast (CEF) tissue cultures[7,8].

Recently, using methods previously described[8–10], we observed the production of antibodies specific for avian leukosis in the serum of a rhesus monkey (kindly supplied by F. J. Rauscher) which developed tumours after inoculation at birth with the Schmidt–Ruppin (*SR*) strain of RSV. This monkey, the offspring of a healthy young mother, was bled for a serum sample when less than 24 h old and then inoculated subcutaneously over both the right and left lower back with 0·5 ml. of *SR*-RSV prepared from chicken wing web tumours[8].

A small subcutaneous tumour was first noted at the site of injection on the right side 21 days after inoculation of the virus. Several subcutaneous tumours afterwards developed and grew progressively at both inoculation sites. Tumours which developed on the left side were excised 7 days after they were first noted. An attempt to establish cells from the excised tumours in tissue culture was unsuccessful. Tumours recurred rapidly at the site of the operation and were readily detectable 7 days after excision. In a second attempt to establish tumour cells in tissue culture, sub-total excisions of the tumours on both sides were performed 85 days after their onset. Cells from this biopsy were successfully grown in tissue culture and were carried through sixteen subpassages (cultures established and carried by C. H. Calisher). Postoperatively, we noticed at the excision sites the formation of firm subcutaneous masses which were indistinguishable from tumour tissue by palpation, but these regressed rapidly and left no gross evidence of tumours 6 weeks later.

Five to six weeks after the onset of tumours, CF antibodies appeared in the monkey serum which reacted with *SR* strain hamster tumour antigens and the antigens found in chicken tumours induced by both *SR* and Bryan strains of RSV but not with the virus-induced hamster tumour antigens of *SV*40 virus, polyoma virus or adenovirus types 7 and 12. The monkey sera with the highest antibody titres against RSV hamster and chicken tumour antigens also reacted specifically with antigens present in CEF cultures of both resistance-inducing factor (RIF) and avian erythroblastosis virus (AEV), but all sera were negative when tested against cell pack preparations of uninoculated CEF cultures used at the same dilutions as the avian leukosis antigens. The progress of specific CF antibody formation is illustrated in Table 1 which shows the reactions of representative serial sera tested against 4–8 units of the specific antigens listed. Antibody titres generally increased during the period of tumour residence but fell to undetectable levels within 3 weeks of subtotal excision and subsequent regression of the tumours. Three of the sera with high CF antibody titres were negative at 1 : 10 dilution when tested for neutralizing antibodies against both *SR* and Bryan strains of RSV. Ten months after excision, tumours did not recur, and serum taken at that time did not react in CF tests with RSV antigens.

Despite the development of CF antibodies, we were unable to demonstrate CF antigens in a 20 per cent extract of tumour tissue excised from this monkey when it was tested at 1 : 2 dilution versus three of the highest titred homologous monkey sera or against standard

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