and Strott[9] indicates that an androgen-independent mechanism is responsible for the post-castrational restoration of androgen-receptors. Preliminary observations (unpublished) show that about 30% reduction in T-binding is effected by a 100-fold excess of DHT and vice versa, in both epididymis and the accessory glands. This may be construed as evidence for an apparent overlap existing between T-specific and DHT-specific binding sites. 5α-androstanediol inhibits labeled DHT-binding, probably due to its conversion to DHT by 3α-reductase. These results indicate that the androgen response created in a tissue is a net result of the combined action of 2 or more androgen metabolites.

Wilson[10] demonstrated an interesting correlation that exists between embryonic differentiation and metabolism of testosterone by different organs of the reproductive system. The Wolffian duct which differentiates to form the epididymis, ductus deferens and seminal vesicles develops the capacity to metabolize testosterone very late during the process of differentiation while the urogenital sinus which later undergoes differentiation to produce the prostate has the T:DHT equilibrium shifted towards the latter. The role of T as an essential factor for Wolffian duct differentiation is thereby indicated while more of DHT than of T is involved in the development of urogenital sinus. It is interesting to note that the fully differentiated epididymis retains a greater amount of testosterone than that done by the other 3 tissues. The ductus deferens and the seminal vesicles at the same time show a metabolite pattern similar to that of the prostate, a urogenital sinus derivative. The significance of this phenomenon is yet to be ascertained.

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PRO EXPERIMENTIS

A method for the determination of free neuraminic acid split from red blood cell receptors by attached Newcastle disease virus during simultaneous elution and hemolysis

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Summary. Determination of free neuraminic acid in chicken red blood cell (RBC) hemolysate becomes possible after deproteinization of the hemolysate by ethanol-chloroform followed by removal of the solvents by evaporation. This procedure permits the determination of in situ neuraminidase activity of virions preadsorbed on RBC receptors when the virus elution and hemolysis proceed simultaneously.

The interaction of myxoviruses with RBC includes attachment of virions to cell receptors at 4 °C and their elution at a temperature optimum of 37 °C[1,2]. The latter process is due to enzymatic action of viral neuraminidase which splits the N-acetyl-neuraminic acid (NANA) moiety from cell receptor glycoproteins[3-8]. However, the question arises whether there is a quantitative correlation between virus neuraminidase (Nase)activity in vitro (using appropriate substrate) and in situ Nase activity of the virus preadsorbed on RBC at 4 °C and eluting at 37 °C. The experimental approach to the problem is based on direct determination of free NANA accumulated in eluate as a result of in situ Nase action of the preadsorbed virus splitting NANA from RBC receptors[8]. However, this approach meets with an obstacle when paramyxoviruses, inducing hemolysis (Newcastle disease virus, in particular), are used. Preliminary data have suggested that in this case hemoglobin accumulated in eluate as a result of hemolysis interferes with the thiobarbituric method of determination of NANA accumulated in the same eluate as a result of Nase activity of eluting virus. This was demonstrated by mixing hemoglobin obtained from osmotic hemolysis of chicken RBC with pure NANA (figure 1). In the present paper, a method for determining free NANA accumulated in the NDV-RBC system as a result of elution which proceeds simultaneously with hemolysis is described.

Materials and methods. The avirulent strain 'Queensland' of NDV, grown in embryonated eggs was used. The virus was partially purified by differential centrifugation at 5000 × g and at 50,000 × g. The pellet was resuspended in 1/20 of the original volume, then treated with fluorocarbon 113 (1:1 v/v) for 1 min and centrifuged at 7500 × g for 10 min. The virus in the upper phase was used. The virus was adsorbed on fowl RBC (15% final concentration) for 30 min at 4 °C. The cells were centrifuged at 4 °C, resuspended in cold saline and immediately transferred to 37 °C. Samples were taken at intervals and centrifuged immediately in an Eppendorf centrifuge at 12,000 rpm for 1 min at 4 °C. Eluted virus, free NANA and hemoglobin were determined in the supernatants. Eluted virus was determined by hemaggluti-

Recovery of NANA from mixtures with different concentrations of hemoglobin

Red blood cell concentration (%)	Hemoglobin (A_{540})	N-acetyl-neuraminic acid recovered (A_{549}*)
0**	-	0.63
1	1.45	0.61
3	4.35	0.63
5	7.9	0.66
8	12.6	0.64
12.5	20.7	0.63

NANA (25 μg) was added to each hemolysate preparation and treated with ethanol-chloroform as described above. * Values after subtracting A_{549} values of hemolysate preparations without NANA. ** Untreated control-NANA in water.

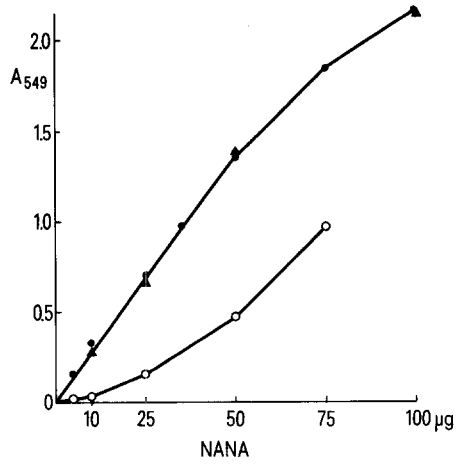

Fig. 1. Effect of hemoglobin on NANA determination. Closed circles, NANA in water; Open circles, NANA in hemolysate prepared by placing RBC (7.5%) in hypotonic solution; Triangles, NANA recovered after treatment of hemolysate with ethanol-chloroform as described above.

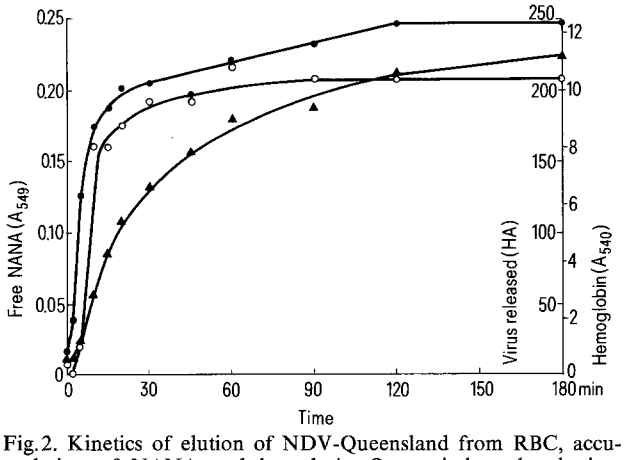

Fig. 2. Kinetics of elution of NDV-Queensland from RBC, accumulation of NANA and hemolysis. Open circles, eluted virus (HA); Closed circle, free NANA; Triangles, hemolysis.

nation test with 1% chicken erythrocytes. Hemoglobin was determined spectrophotometrically at 540 nm. NANA was determined by the Warren method[9] modified by Aminoff[10]. *Results and discussion.* In order to eliminate hemoglobin present in eluate, supernatant samples were deproteinized by the method of Tsuchihashi[11] modified for high concentrations of hemoglobin as follows. An aliquot of 0.24 ml of ehtanol-chloroform (2:1) was added to 0.5 ml supernatant. The protein was sedimented by centrifugation and alcohol-chloroform solvent was then removed by evaporation in vacuo and by heating at 70 °C. The validity of this method for sucessive determination of NANA was demonstrated by complete recovery of NANA determined after its mixing with hemolysates in various ratios (figure 1 and table). As can be seen, neither different concentrations of NANA (up to 100 µg, figure 1), nor different concentrations of hemoglobin (table) in NANA-hemolysate mixtures influenced the values of NANA determined after the deproteinization procedure. This method was then applied to the direct determination of NANA in the NDV-RBC system. It can be seen (figure 2) that the accumulation of NANA in the eluate can be reliably detected during the simultaneous processes of hemolysis and elution. The kinetics of NANA accumulation is correlated with the kinetics of elution. Thus, it is proposed that the detected NANA is accumulated in eluate as a result of in situ Nase action of eluting virus on NANA-containing RBC receptor substrate. This in situ action of the enzyme, contrary to its in vitro activity towards a soluble substrate, may be strongly dependent on steric conditions created between enzymatic sites (hemagglutinin-neuraminidase glycoprotein subunits) of attached virions and NANA-containing receptor sites of RBC. This would cause either facilitation or hindrance for the in situ Nase action. The actual outcome depends on the degree of congruence between viral and receptor sites and, hence, ultimately on the arrangement of the supercapsid viral subunits in the viral envelope. As the latter may be a particular NDV strain-specific property, corresponding studies using various NDV strains differing in their virulence are now in progress.

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A technique for sterile culture of fresh water sponges

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Summary. By treating the gemmules of freshwater sponges successively with hydrogen peroxide and with sodium hypochlorite in suitable concentrations, we obtained cultures that were free from any bacterial or fungal contaminant. This technique provides a useful tool for further studies on metabolic and antibiotic activities, and of the behaviour of isolated cells cultivated in artificial media.

Fresh-water sponges provide excellent models for the experimental study of cell differentiation and behaviour. Indeed, their organization bears more resemblance to a society of cells than to a stable tissue, and the few distinct cell-types are highly motile.

In previous work[2] we have shown that it is possible to inhibit specifically the differentiation of 1 cell type, viz. the choanocytes by hatching the gemmules in the presence of hydroxyurea. Such sponges, devoid of any aquiferous system, are reduced to a closed dermal envelope stretched on