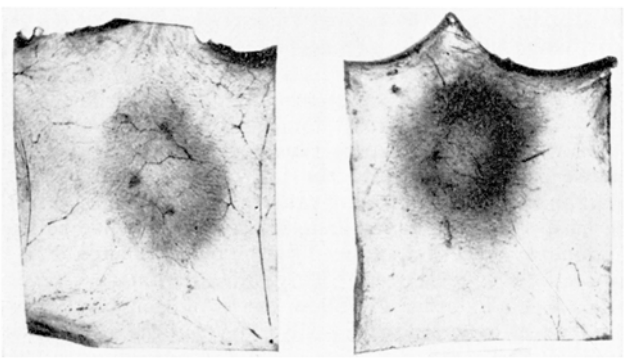
on the inner side of the skin. The muscles, liver and intestine of the operated animals were also more distinctly coloured than those of the controls.

Calcemia in the parathyroidectomized animals was 10.0 ± 1.3 mg%, in the controls 10.8 ± 1.2%; these normal values in the operated animals were somewhat surprising, though, as has been noted[1,2], tetany does not develop in female rats on a normal diet. As the calcium content of our diet was very high (2.9%) this fact might explain these results. It is extremely unlikely that accessory parathyroids would be present in all the operated cases.

Pontamine-Blue concentration in the serum was also determined in order to exclude the possible colour differences in the operated and control rats originating from different stain concentrations in the blood: no statistically significant differences were observed.

In the second experiment the skin of the left side of the abdomen of 29 rats (15 controls and 14 parathyroidectomized 15, 30, and 60 days before) was fixed in Bouin's fluid. Paraffin sections were stained with Toluidine-Blue (Grübler). A 1/5000 solution of the dye was prepared with Mc Ilvaine's buffer (pH 5.4) diluted 1/10.



A B

Spreading of the colour, 1 h after the intradermal injection of rat serum. Inner side of the skin of the abdomen. – (A) control rat; (B) parathyroidectomized rat.

The development of metachromasia was followed microscopically for up to 1 h on several sections of the skin of the operated and control rats coloured simultaneously.

In the control rats, metachromasia of the derma was present in all cases after 12 min; in 13 cases the metachromatic stain intensified reaching an optimum after 20 min; in the other 2 cases, the optimum was reached after 30 min.

Of the operated rats, in only 10 cases was metachromatic staining evident after 12 min and in the other 4 cases only after 20 min; an optimal coloration after 20 min was reached in 6 cases, while the remaining 8 cases only reached this stage after 40 min. These results show that metachromasia of the derma in the operated animals develops less easily than in the controls, indicating structural changes of the mucopolysaccharides. The minor differences can perhaps be explained by the different lengths of time since the operation, or by the presence of accessory parathyroids in some of these animals.

Because of the normal calcium level in the blood of the operated rats, we are inclined to believe that mucopolysaccharide changes, not only of the organic bone matrix but also of the other connective tissues, are primarily related to the parathyroid function[11].

Riassunto. Nei ratti femmine paratiroidectomizzati si osserva un aumento della permeabilità vasale ed una modificazione della colorazione metacromatica del derma, indipendenti dalla concentrazione del calcio nel sangue. Tali variazioni vengono attribuite a modificazioni strutturali dei mucopolisaccaridi della sostanza fondamentale del connettivo.

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Infectious RNA from Ranikhet Disease Virus and its Preservation with Lipid Treatment

Infectious RNA have been isolated from a number of simple plant and animal viruses by the phenol extraction procedure[1,2]. We now report the isolation of infectious RNA, by this method, from Ranikhet disease virus[3] (Indian strain of NDV), a virus belonging to the more complex Myxovirus group. Preliminary experiments indicated that the infectious viral RNA is extremely susceptible to its environment and, unlike the virus itself, loses its infectivity on dilution, variation in environment temperature, storage and contact with ribonuclease. The high lipid content of this virus (ca. 50%) led us to investigate any preservative action lipids might have on the RNA. It was found that treatment of the RNA, immediately after isolation, with viral or mouse brain lipids, resulted in the RNA being able to retain its infectivity on dilution and even on treatment with ribonuclease.

The virus material used in the first series of the present experiments was freshly harvested allantoic fluid from chick-embryos infected with Ranikhet disease virus, clarified by centrifugation at 1500 rpm. The need to establish the identity of the isolated RNA necessitated the use of purified virus. This was obtained by adsorption of the virus from infective allantoic fluid on to aluminium phosphate gel at pH 6 at 0°C, elution at pH 8 at 37°C and isolation by ultracentrifugation at 40000 rpm for $2^1/_2$ h (Miller and Schlesinger procedure[4] modified by O.P.B. and Dr. Nitya Nand). The purified virus was then resuspended in $M/25$ phosphate buffer pH 6. The isolation of

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RNA was carried out at 0–5°C. Virus suspensions were extracted thrice for 7 min with equal volumes of phenol saturated with water. The phases were separated each time by centrifugation (1500 rpm, 5 min) and the aqueous layer freed of phenol by washing six times with peroxide-free ether. Residual ether was removed from the RNA-containing aqueous solutions by bubbling sterile air. The ultra-violet spectrum of the RNA solutions obtained from purified virus had λ_{max} 263 mμ, λ_{min} 238 mμ and E_{263}/E_{285}, 1.8–1.9 indicating the persistance of protein matter. The solutions, however, did not give positive biuret colour tests. Infectious RNA from other viruses also are known to carry small quantities of strongly bound protein matter. As is suggested by electron microscope studies[5], it is possible that these intimately bound proteins are involved in holding the RNA strands in their appropriate positions.

Virus and mouse brain lipids were obtained by extraction with ether over 15 min and washing the ether extracts thrice with phosphate buffer pH 7. For treating the virus RNA with lipids, the RNA solutions obtained after the removal of phenol with ether were shaken with ether extracts of an equal volume of virus suspension or of 5 mg mouse brain per ml of virus suspension and the ether removed by bubbling sterile air through the solutions for 20 min.

All the RNA solutions possessed no haemaggluting activity. For investigation of their infectivity, the pH of these solutions was adjusted to 7.2 and stationary CAM cultures were infected as described earlier[6], with (i) undiluted RNA solutions, (ii) RNA solutions diluted with nutrient medium, (iii) undiluted RNA treated with ribonuclease (0.002 mg/ml) for 20 min at 5° and (iv) undiluted RNA solutions stored for 5 days at – 20°C. In the cases of (i), (iii) and (iv) the cultures were infected with 0.1 ml/culture, and the nutrient medium (0.9 ml) was added 1 h later. In the case of (ii) 1 ml/culture was employed. HA-titres of the culture fluids were then determined after 48 h. Infectivity in chick-embryos was determined according to the procedures described previously[7]. The table summarises the results of the infectivity experiments with purified virus RNA. Entirely analogous data were obtained in the earlier experiments with RNA-containing solutions obtained from infective allantoic fluid, except that the infectivity of these solutions was somewhat greater.

Undiluted RNA solutions were able to infect CAM cultures. Virus obtained by serial passage of the culture fluids was identical with the original virus. Dilution of RNA solutions, ribonuclease treatment or storage at – 20°C resulted in complete loss of infectivity. Lipid-treated RNA, however, infected both CAM cultures and chick embryos. Infectivity was not lost on dilution or ribonuclease treatment but was completely lost on storage at – 20°C. The contention, that the RNA solutions now investigated contained no intact or reconstituted virus, is supported by the overall behaviour of these solutions and the ultra-violet spectrum of the RNA solutions prepared from purified virus. Evidence has, therefore, been presented suggesting that the RNA, as in the case of the simpler viruses, carries the genetic information of Ranikhet disease virus, a virus of the more complex myxo-virus group[8].

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[8] The authors are grateful to Drs. M. L. Dhar and B. N. Singh for their encouragement.

Infectivity of RNA from Ranikhet disease virus

No.[a]	Material tested	Dilution	Infectivity in CAM cultures[b] % infected	HA. titre (mean-log value)	Infectivity in chick embryos[c] % infected	% lesions	HA. titre (mean-log value)
1.	RNA	0	20	0.4	0	0	0
		10^{-1}	0	0	—	—	—
		10^{-2}	0	0	—	—	—
		10^{-3}	0	0	—	—	—
2.	RNA treated with RNase	0	0	0	—	—	—
3.	RNA stored at —20°C for 5 days	0	0	0	—	—	—
4.	VL treated RNA	0	100	1.2	100	100	2.2
		10^{-1}	100	1.2	100	100	2.1
		10^{-2}	100	1.2	100	100	1.9
5.	VL treated RNA treated with RNase	0	100	1.1	—	—	—
		10^{-1}	100	1.2			
		10^{-2}	100	1.1			
6.	VL treated RNA stored at —20°C for 5 days	0	0	0	—	—	—
7.	MBL treated RNA	0	100	1.3	100	100	2.2
		10^{-1}					
		10^{-2}					
8.	MBL treated RNA treated with RNase	0	100	1.2	100	100	2.1
		10^{-1}	100	1.3	100	100	2.1
		10^{-2}	100	1.2	100	100	2.2
9.	MBL treated RNA stored at —20°C for 5 days	0	0	0	—	—	—

Abbreviations: RNA, infective RNA obtained after phenol extraction of purified Ranikhet disease virus; VL, virus lipid; MBL, mouse brain lipid; RNase, ribonuclease.

[a] Each experiment performed at least twice, mean value given.
[b] See [6].
[c] Four chick embryos employed for each fraction; % infected = percentage of embryos infected; % lesion = lesion score[7]-fully infected, 100; uninfected, 0.

Zusammenfassung. Aus Ranikhet-Virus (dem indischen Stamm des Newcastle disease virus) wurde eine infektiöse Ribonukleinsäure isoliert. Diese ist sehr empfindlich gegen Umweltseinflüsse. Verdünnung, Lagerung bei – 20°C und Behandlung mit Ribonuklease führen zum Verlust der Infektivität. Nach Behandlung der isolierten Ribonukleinsäure mit Viruslipid oder Lipid aus Mäusegehirn bleibt die Infektivität aber auch nach Verdünnung oder sogar nach Behandlung mit Ribonuklease erhalten.

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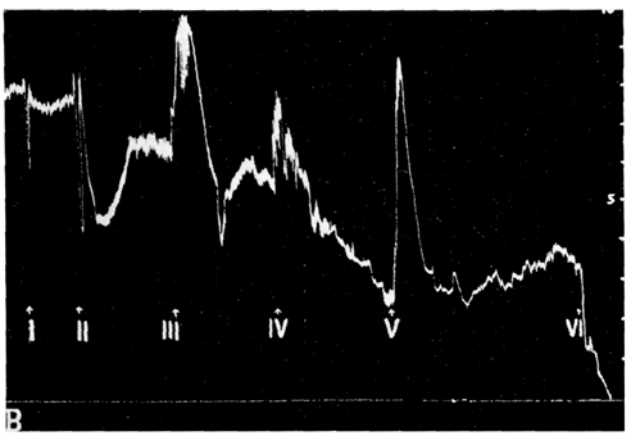
Central Drug Research Institute, Lucknow (India), August 7, 1962.

On the Presence of Adrenaline-Sensitive Receptors at the Cerebral Cortex of the Rabbit

Previous investigations have shown that the topical application of adrenaline to the cerebral cortex of the rabbit elicits an immediate blood pressure rise, and that this reaction is susceptible of undergoing significant changes under the influence of psychotropic substances[1]. Numerous experimental approaches have been devised in an attempt to determine the nature (central or peripheral) of that response. It has thus been demonstrated that size and duration of the cortically induced pressor effects closely depend on the area treated. They are optimal after median fronto-parietal application (parietal and ventral parts of the area praecentralis agranularis), smaller and more transient on the frontal (area praefrontalis granularis), and generally negligible on the temporal and occipital regions (area striata, area spleniaris), though there are no particular differences in the vascular supply between these zones. It has further been noticed that the hypertensive reaction gradually increases after repeated applications to the same area, and that such a contact has a sensitizing effect on subsequently treated cortical fields, even on those which were primitively refractory to the adrenergic transmitter[2]. The cardiovascular effect initiated by cortical administration could be differentiated from that produced by intravenous injections of the same substance, through the injection of a massive adrenaline dose (0.75 to 1.0 mg) which led to a biphasic antagonistic modification of both responses (increase of the intravenous and decrease of the cortical effect in the first, progressive increase of the cortical and definite decrease of the peripheral action, in the second phase of the experiment)[3]. It has also been found that the cortically induced hypertension disappears after surgical disconnection of the treated area or destruction of the anterior hypothalamus. In the latter case cerebro-cortical applications continued to provoke oxytocic manifestations on the uterus *in situ*[4]. These data already pointed to the existence of a limited cortical region upon which adrenaline may exert an adequate stimulus.



Rabbit blood pressure: Animal anaesthetized with ethyl-urethane and injected with Regitine (1 mg/kg). – (I): intravenous injection of 10 µg of adrenaline bitartrate, (II) and (VI): i.v. injection of 30 µg of adrenaline bitartrate, (IV): i.v. injection of 0.5 U of Pitressin, (III) and (V): topical application of a 5% solution of adrenaline bitartrate to the cerebral cortex.

In view of possible physiological or physio-pathological implications of this reaction, it seemed of interest to accumulate further evidence of its essentially central character by completing and controlling these observations through the use of discriminating pharmacodynamic procedures based on the action of some structurally different 'adrenergic blocking agents'. Piperazone (piperidomethyl-3-benzodioxane), Regitine (2(N-*p*-tolyl-N-*m*-phenylamino-methyl) imidazol), and Dibenamine (N.N. dibenzylchloro-ethylamine) were selected. The former two drugs have rapid, but short effects, the latter produces retarded, but long-lasting actions, all three being supposed to attack essentially peripheral receptor sites.

The assays are carried out on male adult rabbits under light (intravenous) urethane anaesthesia. The hemispheres are exposed, the dura is opened, and mean blood pressure measured via a cannula in the left femoral artery. The cortex is carefully rinsed with body-warm Ringer until complete disappearance of all traces of blood. Then a very small piece of filter-paper (3 to 5 mm²), moistened with a freshly prepared 5% solution of adrenaline bitartrate in Ringer is placed on the left fronto-parietal zone. After a short contact (generally not more than 30 sec), the systemic blood pressure rises, maintains a maximum for some minutes, and then reaches again its initial level.

However, in the following experiments, conducted during a period of reduced cortical sensitivity, applications were made to the fronto-parietal areas of both sides, and the contact was prolonged up to 1 min. Control assays had previously shown that the observed pressor effects were not due to the presence of a high concentration of the base or the tartrate radical, nor to the acidity of the solution. The hypertensive effects initiated by topical application corresponded to those engendered by intravenous injections of 10 to 20 µg of adrenaline bitartrate. The

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