		Normale Tiere	Nach Nephrektomie		
			8 h	24 h	48 h
Plasma	Na^+	14,30 ± 0,12 (64)	14,51 ± 0,42 (5)	13,48 ± 0,29 (5)	13,75 ± 0,23 (5)
mAeq.%	K^+	0,54 ± 0,01 (68)	$0{,}71^{**}$ ± 0,06 (5)	$0{,}72^{**}$ ± 0,04 (5)	$1{,}02^{***}$ ± 0,06 (4)
	Cl'	10,92 ± 0,08 (68)	$9{,}26^{***}$ ± 0,45 (5)	$9{,}83^{**}$ ± 0,45 (4)	$6{,}00^{***}$ ± 0,81 (5)
Erythro-	Na^+	1,87 ± 0,05 (64)	$3{,}18^{***}$ ± 0,40 (5)	$2{,}40^{**}$ ± 0,18 (5)	$2{,}66^{***}$ ± 0,41 (5)
zyten	K^+	10,39 ± 0,09 (68)	9,72 ± 1,33 (5)	$7{,}22^{***}$ ± 0,37 (5)	$5{,}50^{***}$ ± 0,31 (5)
mAeq.%	Cl'	5,22 ± 0,07 (68)	$3{,}82^{***}$ ± 0,90 (5)	$4{,}42^{**}$ ± 0,19 (5)	4,84 ± 0,41 (5)

fen untersucht[1]. Da Schafe sich aber von Menschen, Hunden und Ratten im Verhalten der Plasmaelektrolyte nach Nephrektomie wesentlich unterscheiden[2], erschien die Untersuchung der Verhältnisse bei der Ratte angebracht.

Methodik. 48 mit Latz-Rattenfutter (Firma Latz, Euskirchen/Rheinland) ernährte Ratten, die Wasser *ad libitum* erhielten, wurden zunächst einseitig nephrektomiert: 3 Wochen später wurde die andere Niere in Äthernarkose exstirpiert. Vorher festgelegte Gruppen von je 8 Tieren wurden 8, 16, 24, 32, 40 und 48 h nach der Totalnephrektomie in leichter Äthernarkose aus den Carotiden ausgeblutet. Blut wurde über Heparin aufgefangen und sofort zentrifugiert. Plasma und Erythrozyten wurden getrennt mit 0,75 *n* HNO_3 24–48 h lang extrahiert. Im Extrakt wurden Na^+ und K^+ flammenphotometrisch, Cl' merkurimetrisch[3] bestimmt.

Ergebnisse und Diskussion. Die Ergebnisse der Analysen von Plasma und Erythrozyten 8, 24 und 48 h nach der Nephrektomie sind in der Tabelle den bei normalen Tieren gefundenen Werten gegenübergestellt.

Angegeben sind jeweils die Mittelwerte ± $s\bar{x}$, in Klammern die Tierzahlen. Die Signifikanz der gefundenen Unterschiede der Mittelwerte von den Normalwerten ist durch * = $P < 0{,}05$; ** = $P < 0{,}01$ und *** = $P < 0{,}001$ bezeichnet.

Die Hyperkaliämie nach Nephrektomie ist bei Ratten nicht ganz so gross wie bei anurischen Hunden[4] und Menschen. Besonders auffällig ist dagegen der Abfall des K^+-Gehaltes der Erythrozyten nach Nephrektomie, der nach 24 h etwa ¾, nach 48 h etwa noch die Hälfte des Ausgangswertes beträgt. Das von den Erythrozyten nicht mehr festgehaltene K^+ muss sich in einem weit grösseren Flüssigkeitsvolumen als dem Gesamtplasma verteilen, da sonst die Hyperkaliämie viel stärker ausgeprägt sein müsste. Die in die Erythrozyten eindringende Na^+-Menge ist kleiner als die ausgetretene K^+-Menge. Das Eindringen von Na^+ in die Erythrozyten führt nicht zu einem Absinken des Plasmaspiegels; im Hinblick auf die wahrscheinliche Zunahme des Plasmavolumens bedeutet das ein Übergehen von Na^+ aus den Körpergeweben ins Blut. Die Veränderungen des Plasma-Cl'-Spiegels sind wahrscheinlich Ausdruck der Zunahme des Extrazellulärraumes.

Grundsätzlich ähnliche, aber reversible Veränderungen wie bei nephrektomierten Ratten wurden bei 2 Kindern mit akuter Anurie erhoben. Die mitgeteilten Ergebnisse werden in ausführlicherer Darstellung der Schriftleitung der Klinischen Wochenschrift zur Publikation eingereicht.

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Pharmakologisches Institut der Universität Mainz, 24. August 1956.

Summary

After two-stage nephrectomy in rats the potassium concentration in the red blood corpuscles (RBC) decreases from 10·6 ± 0·3 to 5·5 ± 0·3 mEq per 100 ml of RBC within 48 h. The decrease is accompanied by a much smaller increase in the plasma potassium concentration; the hyperkaliemia in nephrectomized rats is less pronounced than in nephrectomized dogs or anuric humans. Na^+ in RBC increases by about 44% after nephrectomy; while there is only a very slight decrease of Na^+ in plasma. Plasma chlorides drop from 10·92 ± 0·08 mEq/100 ml of plasma to 6·00 ± 0·81 mEq % within 48 h after nephrectomy. RBC chlorides tend to increase again after an initial drop from 5·22 ± 0·07 to 3·82 ± 0·90 mEq% within the first 8 h.

Changes in Antigenicity of Rats Spleen Cells Nuclei Resulting from Total-Body X-Irradiation

The present communication concerns the effect of X-ray irradiation upon the antigenicity of nuclei of spleen cells of rats, as well as the possible role played by deoxyribonucleic acid and deoxyribonucleoprotein in cells nuclei antigenicity.

The spleen cells nuclei have been used in experiments. Young adult rats were exposed to lethal dosis. For this purpose a 'Siemens' Roentgen apparatus operated at 220 kV, 14 mA, 3 F A1, giving a dose of 150 r/mm at the target distance of 40 cm, was used. The animals were sacrified 24 h after irradiation, and the spleen removed in the cold. The nuclei from X-irradiated and normal rats were isolated according to the method described by

[1] H. HARRIS, I. R. MCDONALD und W. WILLIAMS, Austr. J. exp. Biol. med. Sci. *30*, 33 (1952).

[2] H. HARRIS, I. R. MCDONALD und W. WILLIAMS, Austr. J. exp. Biol. med. Sci. *30*, 33 (1952). – I. R. MCDONALD, D. A. COATS und J. MUNRO, Austr. J. exp. Biol. med. Sci. *32*, 275 (1954).

[3] G. KUSCHINSKY und H. LANGECKER, Biochem. Z. *318*, 164 (1947).

[4] H. E. HOFF, P. K. SMITH und A. W. WINKLER, J. clin. Invest. *20*, 607 (1941).

Spleen nuclei used for immunization	Material used for absorption	Dilutions of rabbit antisera: 1 in —											
		1	2	4	8	16	32	64	128	256	512	1024	2048
		Antiserum No. 1											
SNNR . .	unabsorbed serum	+	++	++	v	v	++	+	+	+	–	–	–
	DNA	0	+	+	+	+	–	–	–	–	–	–	–
	DNAP	0	+	+	+	–	–	–	–	–	–	–	–
		Antiserum No. 2											
SNNR . .	unabsorbed serum	–	+	+	++	v	v	v	++	+	+	+	–
	DNA	0	+	+	++	+	+	–	–	–	–	–	–
	DNAP	0	–	–	–	–	–	–	–	–	–	–	–
		Antiserum No. 3*											
SNXIR . .	unabsorbed serum	–	+	++	++	++	+	+	–	–	–	–	–
	DNA	0	+	+	++	+	+	–	–	–	–	–	–
	DNAP	0	+	+	++	+	–	–	–	–	–	–	–
		Antiserum No. 4*											
SNXIR . .	unabsorbed serum	+	+	++	++	+	+	–	–	–	–	–	–
	DNA	0	+	+	+	+	–	–	–	–	–	–	–
	DNAP	0	+	+	+	–	–	–	–	–	–	–	–

All tests were done with SNNR. Controls: (a) titration of normal rabbit serum against SNNR nuclei in the cold was negative; (b) suspension of SNNR nuclei in saline kept in the cold did not show agglutination. The absorptions were done with DNA and DNAP isolated from normal spleen cells nuclei.

* Unabsorbed and absorbed antisera No. 3 and 4 were also titrated against SNXIR nuclei, but no difference in the titers could be found as compared with those obtained with SNNR.

HOGEBOOM *et al.*[1]. Deoxyribonucleic acid (DNA), highly polymerized, was obtained by the method of ZAMENCHOF *et al.*[2]; deoxyribonucleoprotein (DNAP) was extracted by the method of BUTLER *et al.*[3], and the technique of MIRSKY and POLLISTER[4] was employed for the separation of ribonucleoprotein (RNAP). DNA and DNAP were prepared from normal spleen cells nuclei, and RNAP from normal total organ. The concentrations of DNA, DNAP and RNAP were determined by the following methods: CERIOTTI's method[5] for DNA, SAKAGUCHI's method[6] for DNAP and biuret method[7] for RNAP.

Male albino rabbits weighing from 2 to 2·5 kg were immunized. Their sera were titrated for antibody content prior to immunization. One group of animals received several injections of a suspension of spleen cells nuclei from normal rats (SNNR), and a second group several injections of a suspension of spleen cells nuclei from X-irradiated rats (SNXIR). Both groups of animals received equal number of spleen cells nuclei. The bleedings for antibody analyses were made ten days after the last injection. The absorption of immune rabbit sera was performed using DNA, DNAP and RNAP. In some cases, the absorptions of immune sera were also made with DNA, DNAP and RNAP obtained from normal rats liver. One volume of immune serum and one volume of DNA, DNAP or RNAP were mixed and left at 4°C for a period of 4 h, followed by a centrifugation at 4°C. The supernatants were used in tests. The agglutination was performed in small tubes (7 × 45 mm). The sera were tested by usual dilution method, using the suspension (80,000 nuclei/mm^3) of SNNR. The incubation period was 16 h at 4°C. The agglutination was read microscopically. All materials used in test were kept in the cold to avoid the denaturation of DNA and DNAP.

The results are partially plotted in the Table. It seems that the SNNR have somewhat higher antigenic power, as compared with SNXIR. The results of absorptions done with DNA and DNAP pointed out that these cell constituents had a pronounced ability to absorbe anti-SNNR antibodies (antisera No. 1 and 2), but only a slight power to absorb anti-SNXIR antibodies from the sera No. 3 and 4. It seems reasonable to attribute this difference to the fact that SNNR, injected to animals, produces antibodies differing from those produced by SNXIR. From that standpoint it can be assumed that a difference in antigenicty and/or antigenic structure exists between SNNR and SNXIR. The DNA, DNAP and RNAP from normal liver cells cross-react with the anti-SNNR antibodies.

These results suggest two possible explanations regarding the inability of DNA and DNAP (isolated from normal spleen cells nuclei) to absorb anti-SNXIR antibodies. The first explanation would be that the SNXIR nuclei contain altered DNA and DNAP. Therefore, they are not capable of inducing the production of anti-spleen-nuclei antibodies which in their molecules have functional groups reacting with normal DNA and DNAP.

[1] G. H. HOGEBOOM, W. C. SCHNEIDER, and M. J. STRIEBICH, J. biol. Chem. *196*, 111 (1952).

[2] S. ZAMENCHOF, H. E. ALEXANDER, and G. LEIDY, J. exp. Med. *98*, 373 (1953).

[3] J. A. V. BUTLER, P. F. DAVISON, D. W. F. JAMES, and K. V. SHOOTER, Biochem. biophys. Acta *13*, 224 (1954).

[4] A. E. MIRSKY and A. POLLISTER, J. gen. Physiol. *30*, 101 (1946).

[5] G. CERIOTTI, J. biol. Chem. *198*, 297 (1952).

[6] S. SAKAGUCHI, in *Practical Methods in Biochemistry*, ed. by KOCH and HANKEL, p. 74, 6th Ed. (Williams & Wilkins, Baltimore 1953).

[7] *Practical Methods in Biochemistry*, ed. by KOCH and HANKEL, p. 195, 6th Ed. (Williams & Wilkins, Baltimore 1953).

The second explanation would be that DNA and DNAP probably play an important role in the antigenicity of spleen cells nuclei. Although the evidence regarding the antigenicity of DNA and DNAP is not altogether complete, it can be assumed on the basis of present experiments that DNA and DNAP have at least hapten-like properties.

A full report will be published elsewhere.

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Résumé

Les études immunologiques de l'ADN et l'ADNP, extraits des cellules normales de la rate des rats, ont demontré que ces constituants cellulaires ont au moins la propriété d'haptène.

Amino Acid Composition of Crude and Germinated Guarseed Flour Protein (*Cyamopsis Psoralioides*)

PARIKH[1] has carried out preliminary investigations on the chemical composition of Guarseed (*Cyamopsis Psoralioides*), a cheap legume grown in abundance in this part of the country. The crude Guar, even though it has a high protein content, is not used for human consumption on account of its bitter taste, high crude fibre content and the difficulty in removing the outer coating. The author was successful in easily removing the outer coating and reducing the fibre content by germinating the Guarseeds for two days before processing. An attempt is being made to investigate the nutritive value of the germinated guarseed flour, as a preliminary to which studies were carried out to determine the amino acid composition of the protein of the crude and germinated guarseed flour. The results are recorded in this communication.

Preparation of the guar flour. The guarseeds were obtained from the local market on a large scale, soaked for 6 h in water and washed till the washings were clear (initial washings usually contained a lot of mud and the washings were of a red colour). One portion was dried in the sun, ground in a mill and sieved through a sixty mesh sieve. The other portion was mixed with mud, and sufficient water to keep them wet was added, after which it was allowed to germinate for 48 h. After the period of germination, the seeds were dried in the sun. All the mud was removed by rubbing in a sieve and most of the skin also came away with the mud. They were again washed, dried, ground in a mill and sieved.

Analytical methods. For the determination of the free amino acid contents of the flour, one gram of flour was taken in 50 ml of water, homogenised for ½ h with a glass homogeniser at room temperature and centrifuged. The supernatant was used for the determinations.

For determining the total amino acids, 0·1 g of the flour was refluxed with 10 ml of 6*n* HCl for 22 h on a sand bath, the excess hydrochloric acid was evaporated under reduced pressure, the residue was mixed with water, neutralised to 6·6 pH and again evaporated and the residue thus obtained was taken in 1 ml of 10% isopropanol and used for chromatography. For the determination of amino acids, the method used was the circular chromatographic method of RAO and WADHWANI[2] except that the chromatograms were run thrice.

The Table shows the free and total amino acid composition of crude and germinated Guar flour. It is found that in the case of the germinated Guar flour, it contains a high percentage of amino acids which gives an indication that it may be a good quality protein. Further work is in progress to study the biological value of the germinated guar flour protein.

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Department of Biochemistry, Baroda University, India, October 8, 1956.

[1] J. M. PARIKH, M. Sc. Thesis, M. S. University of Baroda, India (1954).

[2] N. A. N. RAO and T. K. WADHWANI, J. Ind. Inst. Science *37*, 2 (1955).

Amino acid composition of Guar Flour* (Calculated to 16 g of Nitrogen)

	Free amino acid of		Amino acid composition of the hydrolysate of	
	Crude Guar	48 h Germinated Guar	Crude Guar	48 h Germinated Guar
Cystine	–	0·8	0·27	1·20
Lysine	9·1	12·3	10·30	11·80
Histidine	0·1	0·14	0·30	2·40
Arginine	1·2	2·40	2·05	3·00
Aspartic acid	–	12·20	10·20	14·80
Serine	–	1·80	0·50	1·56
Glycine	0·07	1·23	1·25	2·50
Glutamic acid	15·20	24·20	18·57	22·45
Threonine	–	1·90	0·30	2·10
Alanine	0·12	1·20	0·23	1·34
Tryptophane	0·10	0·70	0·31	0·78
Tyrosine	0·50	1·20	0·70	1·78
Valine	5·30	7·50	6·10	8·30
Methionine	3·10	5·60	4·20	5·80
Phenylalanine	–	0·30	1·04	1·22
Leucine	8·20	13·20	16·10	12·50
Isoleucine	2·10	10·80	4·20	10·10

* Nitrogen content of crude Guar flour: 5·6%.
Nitrogen content of germinated Guar flour: 6·6%.