mosaicism) have been made. By this technique, any heterogeneity in respect to catalase activity can be detected, as shown in Figure 1b. Evidently this is not the case in blood of heterozygotes. However, it has been observed that in blood smears from homozygous acatalatics one out of 100–150 red cells (i.e. 0.6–1.0%) behaves as if it contained approximately the same amount of catalase as a normal red cell (Figure 1a). The percentage of apparently normal red cells detectable in blood smears of acatalatic individuals of the families V., B. or G.[7] roughly corresponds to the level of residual catalase activity as measured by Feinstein's perborate method (0.1–1.3% of normal level). This figure, however, also falls within the range of reticulocyte counts of the samples analysed (0.3–1.0%). Therefore it cannot be decided whether residual catalase activity in blood of homozygotes is due to its presence in reticulocytes or to a small fraction of apparently normal red cells.

Additional evidence for even distribution of catalase in blood of heterozygotes has been obtained by chemical measurements of methemoglobin formation at varied rates of $H_2O_2$ generation. In suspensions of acatalatic red cells, $H_2O_2$ is used almost quantitatively for hemoglobin oxidation; but if catalase is present in the system, only a small fraction of hemoglobin is oxidized. Therefore the amount of methemoglobin formed at a given $H_2O_2$-production rate is a function of catalase activity[4,5]. By plotting methemoglobin formation against the rate of $H_2O_2$-generation, curves of different slope and shape are obtained (Figure 2). The difference between curve 2 and 3 is in agreement with the finding mentioned above that in blood of heterozygotes no evidence could be obtained for the existence of two cell populations differing in catalase activity.

The observations presented in Figure 1 and 2 suggest that in blood of heterozygotes (= 'Hypocatalasemia') all red cells exert approximately the same level of catalase activity. The homogeneous distribution of catalase in erythrocytes of heterozygous individuals from all three Swiss acatalasia families is in agreement with the concept that mosaicism in heterozygotes has been found so far only in enzyme defects, which are inherited by the X-chromosomes[1,9,10].

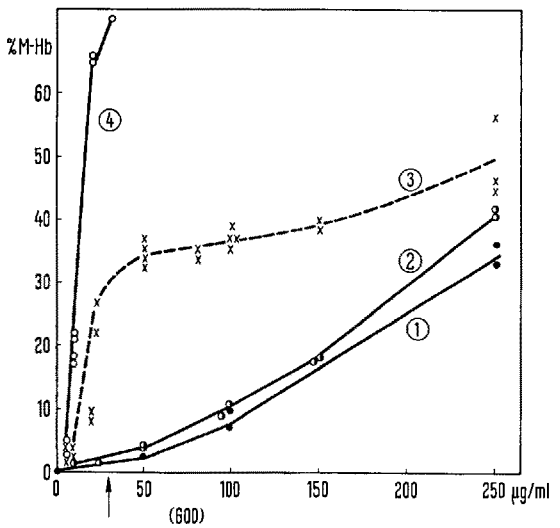


Fig. 2. Methemoglobin formation as a function of the rate of $H_2O_2$ production. Cell suspensions (approx. 1.5 mg Hb/ml) incubated for 30 min at 25°C. For spectrophotometric estimation of methemoglobin the method of FLEISCH[8] has been used. (1) Blood of H.A. (normal), (2) blood of P.V. (heterozygote = hypocatalasaemia; mother of M.V.), (3) 1:1 = mixture of (1) and (4), (4) blood of M.V. (homozygous acatalasia). The arrow indicates the experimental conditions used in the standard procedure (i. e. 30 µg/ml Glucose-oxidase = GOD).

*Zusammenfassung.* Es wird ein Verfahren beschrieben, welches im Blutausstrich katalasehaltige und katalasefreie Erythrocyten zu unterscheiden erlaubt. Dieses beruht auf der sehr ungleichen Methämoglobinbildung bei Inkubierung der Zellen mit Glucose und Glucoseoxidase. Es wird nachgewiesen, dass im Blut homozygoter Defektträger 0,5–1% aller Erythrocyten einen anscheinend normalen Katalasegehalt aufweisen. Im Blut heterozygoter Defektträger konnten keine Anhaltspunkte für das Bestehen zweier Zellpopulationen von verschiedener Kataleseaktivität gefunden werden.

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[7] In a second screening performed in Spring 1965 a third acatalasia family G. was detected in Canton Uri. Three out of ten children are homozygous.

[8] H. FLEISCH, Helv. physiol. Acta *17*, 318 (1959).

[9] O. TÖNZ and E. ROSSI, Nature *202*, 606 (1964).

[10] Acknowledgments: The authors wish to thank Drs. J. ROGGO, Riddes, PD. W. SIEGENTHALER, Zürich and M. JANN, Altdorf, for their kind cooperation in obtaining the blood specimens and Dr. U. BUCHER, Bern, for taking the photographs. This investigation is part of project No. 2879 financed by the Schweizerischer Nationalfonds.

## Renal Function Following Complete Ligation of the Renal Artery

It is well known that after complete ligation of the renal artery some blood still leaves the kidney through the renal vein (HARTWICH[1]; WOLF and HEINSEN[2] etc.). Anatomists maintain that collateral vessels reach the kidney through various branches of the suprarenal and ureteral arteries and the arcus exorenalis, respectively (CORNING[3]). The patchy character of the necrosis after complete ligation supports the functional significance of these collaterals (SHEEHAN and DAVIS[4] etc.). There are, however, no data concerning renal function after occlusion. We endeav-

[1] A. HARTWICH, Z. ges. exp. Med. *69*, 462 (1930).

[2] H. J. WOLF and H. A. HEINSEN, Arch. exp. Path. Pharmak. *179*, 15 (1935).

[3] H. K. CORNING, *Lehrbuch der topographischen Anatomie*, 15. Aufl. (J. F. BERGMANN, München 1923), p. 469.

[4] H. L. SHEEHAN and J. C. DAVIS, J. Path. Bact. *77*, 33 (1959).

oured to elucidate the question by assessing renal function at various intervals after ligation of the renal artery.

Our observations were made on dogs. The left renal artery was ligated. After a certain time had elapsed, the actual experiment was made. The dogs were anaesthetized by chloralose. The left kidney was approached by median laparatomy and the left renal vein was connected by means of a plastic catheter to the external jugular vein. A T-extension of the tube made the direct measurement of renal blood flow (RBF) possible. Urine (U) was collected by means of a ureter catheter; the necessary concentrations of PAH and inulin were maintained by the usual infusions of these substances. Glomerular filtration rate (GFR) was calculated by the formula $C_{in} = RPF \cdot E_{in}$, where RPF stands for renal plasma flow as determined from RBF measured directly and the hematocrit value. $E_{in}$ and $E_{PAH}$ are the extraction ratios of inulin and PAH, respectively.

In the first set of experiments the left renal artery was ligated, while the right kidney remained undisturbed. As a matter of course, no signs of renal insufficiency developed and azotaemia was absent. Data in Table I reveal renal function after 7 to 50 days of complete renal ischaemia. RBF amounts to only a fraction of the control values, filtration and secretion of urine are practically non-existent.

Table I

| No. | Days after ligation of left renal artery | Days after removal of right kidney | RBF | GFR | V | $E_{PAH}$ |
|---|---|---|---|---|---|---|
| | | | ml/min/100 g kidney | | | |
| 77/64 | 7 | – | 48 | 3 | 0 | 0.10 |
| 78/64 | 7 | – | 80 | 2 | 0 | 0 |
| 85/64 | 13 | – | 104 | 4 | 0 | 0.09 |
| 88/64 | 20 | – | 100 | 0 | 0 | 0.07 |
| 91/64 | 20 | – | 85 | 0 | 0.07 | 0.04 |
| 15/65 | 50 | – | 37 | 0 | 0.03 | 0 |
| 116/64 | 161 | 80 | 217 | 25 | 0.86 | 0.30 |
| 35/65 | 106 | 14 | 324 | 39 | 0.48 | 0.58 |
| 47/65 | 126 | 34 | 266 | 43 | 2.71 | 0.66 |
| 77/65 | 123 | 13 | 171 | 34 | 1.71 | 0.77 |
| Control[a] | – | – | 467 | 59 | 0.95 | 0.68 |

[a] P BÁLINT and I. FORGÁCS, Acta physiol. Acad. Sci. Hung. *25*, 203 (1964).

Table II

| No. | Days after ligation of left renal artery | Days after removal of right kidney | NPN mg p.c. | Fate of the animal |
|---|---|---|---|---|
| 33/64 | 47 | 5 | 174 | + |
| 34/64 | 56 | 7 | 256 | + |
| 35/64 | 77 | 7 | 194 | + |
| 56/64 | 108 | 7 | 176 | + |
| 116/64 | 161 | 80 | 104 | E |
| 35/65 | 106 | 14 | 40 | E |
| 47/65 | 126 | 34 | 36 | E |
| 77/65 | 123 | 13 | 38 | E |

+, Spontaneous death. E, actual experiment.

In the second set of experiments, the right kidney was removed at various times after ligation of the left renal artery. As shown in the upper part of Table II, four animals succumbed to renal failure following removal of the right kidney. They exhibited the usual symptoms of renal insufficiency (azotaemia etc.) and survived for 5 to 7 days only, as do dogs after removal of both kidneys.

In four cases, however, our dogs survived nephrectomy (see lower part of Table II). In these cases nephrectomy was performed 81, 92, 92, and 110 days after ligation of the left renal artery, respectively. In all four cases there was a transient azotaemia, but in three dogs NPN values returned to normal. In one case (No. 116/64) azotaemia was maintained; nevertheless, the dog survived without apparent signs of renal failure.

Data concerning renal function of the four surviving animals are presented in the lower part of Table I. It can be seen that RBF and GFR amount to about one-half to two-thirds of the control; urine output is satisfactory and extraction ratios for PAH vary from very low to normal values. It should be stressed that the time of the observation was set arbitrarily. In one case we let the dog survive for 80 days; in the others 13, 14, and 34 days, respectively, elapsed after the right nephrectomy had been performed. We think, however, that dogs showing no signs of azotaemia for two weeks after nephrectomy can be considered as survivors.

The usual histological examinations revealed patchy necrosis alternating with areas without any pathological alterations. In some cases Indian ink or polyvinyl chloride solution was injected into the aorta (technique of MUNKÁCSI[5]); in the surviving cases the collateral vessels could be demonstrated clearly. They entered the kidney from the ureteral arteries and from the arcus exorenalis.

It can be concluded that after complete ligation of the renal artery, despite some remnant of RBF, the kidney ceases to function. If, however, the other kidney is removed about 81 to 110 days after the ligation, the dog can survive without manifesting renal failure. The developing collateral circulation restores the function of the kidney with the occluded artery.

*Zusammenfassung.* Totale Unterbindung der Nieren arterie führt zu fast vollständigem Erlöschen der Funktion der betreffenden Niere. Wird etwa 81 bis 110 Tagnach der Unterbindung die andere Niere entfernt, se können Hunde ohne Symptome von Niereninsuffizieno überleben: die Entwicklung eines Kollateralkreislaufez stellt die Funktion der ischämisierten Niere wieder her. s

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[5] I. MUNKÁCSI, Z. wiss. Mikr. *63*, 352 (1957).