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# Acute Renal Insufficiency in the Rabbit by Glycerol

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The appearance of an acute renal insufficiency in the rabbit, after glycerol injection (10, 13 or 15 ml/kg of a 50 % solution) is investigated. After a 24 hours of intoxication, especially in the ten following days, cylinders, erythrocytes and renal cells appear in the urine sediment. Proteinuria appears after 24 hours and practically disappears after 72 h. Glucosuria persists from 24 hours to 6 days. Haemoglobinuria is intense after 24 and 48 hours and persists slightly about 6 days. Na, K and Cl elimination in urine diminishes clearly in all animals. Plasma K increases in non-surviving animals and does not change in those surviving. Plasma Na does not change in the dying ones, and decreases in those surviving. In non-surviving animals, *p*H, $pCO_2$ and $CO_3H^-$ decrease sharply. In the surviving ones $pCO_2$ decreases clearly after 24 hours, increasing afterwards slowly to normal values. *p*H increases, slightly during the first 48 hours, and then neatly during approximately 6 days. Standard $CO_3H^-$ does not change during the first 48 hours, increasing afterwards during 6 to 7 days. Histologically, the chief lesion is a vacuolar degeneration of the proximal tubule. The possible mechanisms of such alterations are discussed.

Among the causes of acute renal insufficiency we find post transfusional shocks or the «crush-syndrome» (with haemoglobinuria or myoglobinuria as possible determinant factors) and intoxication by heavy metals. More recently, intoxication by ethylenglycol (6) and xylitol (8) has been added to these causes. At present, the treatment of acute renal insufficiency can be applied with much greater effectiveness, though its etiopathogenical mechanisms are not fully known and some factors, like the importance of haemoglobinuria, are discussed. An experimental pattern, known since time ago, is experimental intoxication by mercuric chloride (7), whose characteristics differ fairly (no appearance of haemoglominuria, among others) from many cases present in human

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clinic. An experimental pattern nearer to them is the one studied by CAMERON and FINCKH in 1956 in the rat, by means of intoxication with propanotriol (glycerol). This pattern has been especially studied in some aspects, such as the histological and haemoglobinurical ones, and their intrarenal dynamics (4, 5, 9, 10, 12), though to our knowledge, it has never been studied under other aspects of the general renal functionalism, and especially in what concerns the modifications, it induces in the acid base equilibrium and in plasma and urine ionogram. In this paper, the general characteristics, functional and histopathological, of such an intoxication by glycerol in the rabbit are studied.

## Materials and Methods

*Animals.* White giant rabbits have been utilized, supplied by Massalles (Barcelona), weighing between 3,100 and 3,700 g. The animals have been put into metabolical cages two days before the beginning of the basal period, determinations, period prolonged during a week before their intoxication. They were fed with synthetical diet (Sanders, Tarragona) and water *ad libitum*. In these series no animals with a previous period of dehydratation have been studied.

*Propanotriol (glycerol).* Propanotriol Merck (pa) has been utilized, half diluted with water, as did OKEN *et al.* (12), though others used saline solution (9).

*Intoxication.* The animals have been intoxicated under light ethereal anesthesia, injecting subcutaneously in the lumbar region 10, 13 or 15 ml/kg of the 50 % propanotriol dilution, according to the series.

*Sample collection.* Total urine eliminated in 24 hours was collected, avoiding its mingling with feces, and registering its total volume before obtaining aliquots for further determinations. Blood extractions in anaerobical conditions have been done through the central artery of the ear.

*Determination methods.* Proteinuria has been determined turbidimetrically, precipitating with sulphosalycilic acid. Glucosuria by Benedict. Haemoglobinuria has been determined semiquantitatively by Bencidin test. Sodium and potasium by flame photometer. Chlorine by potenciometer. *p*H, $pCO_2$ and standard $CO_3H^-$ with an Astrup's micro equipment.

## Results

*Toxicity.* A series of 9 rabbits was intoxicated with 10 ml/kg of glycerol dilution, surviving all of them. Another series of 8 was intoxicated with 13 ml/kg, surviving 3 (62,5 % mortality). A final series of 9 rabbits was intoxicated with 15 ml/kg, surviving only two after 11 days (77 % mortality).

*Diuresis.* In the series intoxicated with 10 or 13 ml/kg, there is no oliguria appreciated after intoxication, though a phase of polyuria is observed, much clearer indeed in those animals intoxicated with 13 ml/kg, beginning after 48 hours and lasting two to three days. In the series intoxicated with the higher dose, after 24 and 48 hours, a clear tendency to oliguria appears, which has been corrected in the surviving animals.

Though the number of animals was not very high, survivings does not seem related with the degree of oliguria presented after 24 and 48 hours of intoxicating.

*Morphological data of the urinary sediments.* The urine of rabbits presents normally a high quantity of mineral sediment, constituted chiefly by calcium carbonate. In the urine of intoxicated animals a clear diminution of such a sediment is observed during the days follow-

Table I. *Morphological data of the urine sediment during bassal period and periods post intoxication with glycerol in the rabbit* *.

| Period Post intoxication | Nr. observ./Nr. anim. | Cylinders % pos.** | Erythrocytes % pos.** | Renal cells % pos.** |
|---|---|---|---|---|
| Basal | 142/26 | 0.0 | 0.0 | 0.0 |
| 24 hours | 24/25 | 8.3 | 29.1 | 36.0 |
| 48 » | 18/20 | 27.7 | 26.3 | 44.0 |
| 3- 6 days | 58/15 | 23.9 | 23.2 | 22.8 |
| 7-10 » | 61/15 | 13.5 | 6.5 | 6.6 |
| 11-17 » | 61/14 | 3.2 | 3.1 | 0.0 |

* Taking together all three series of intoxicated animals.
** Expressed in % of its presence in relation with the total of observations realized (independently of its quantity, which is discrete).

ing intoxication, certainly due to a slight urine acidification. The most interesting data pointing to a possible renal injury are the presence or not of cylinders, erythrocytes and renal cells. In table I, their evaluation is presented in the basal period and in periods post intoxication, expressed in % of positivity of their presence in relation to the totality of observations carried out and independently of their quantity. This quantity is always moderated, maximal 2 to 5 erythrocytes by microscopic field at × 500, and nearly the same for the other elements. As it is shown on the table, after intoxication an evident increase of the positivity of these three elements appears, which reaches its maximum about 48 hours, remaining in approximate proportions until 6 days, after which it decreases slowly, reaching from 11 to 17, minimal or negative values.

*Proteinuria, glicosuria and haemoglobinuria.* In the three lots of animals, proteinuria and glicosuria are observed 24 hours post intoxication (fig. 1). Proteinuria presents its top peak exactly at 24 hours and after 72 hours it has nearly disappeared in two of the lots. Glicosuria does not present such a pronounced peak, though its maximal value is maintained for 6 days, disappearing afterwards rather soon.

Haemoglobinuria presents a maximal

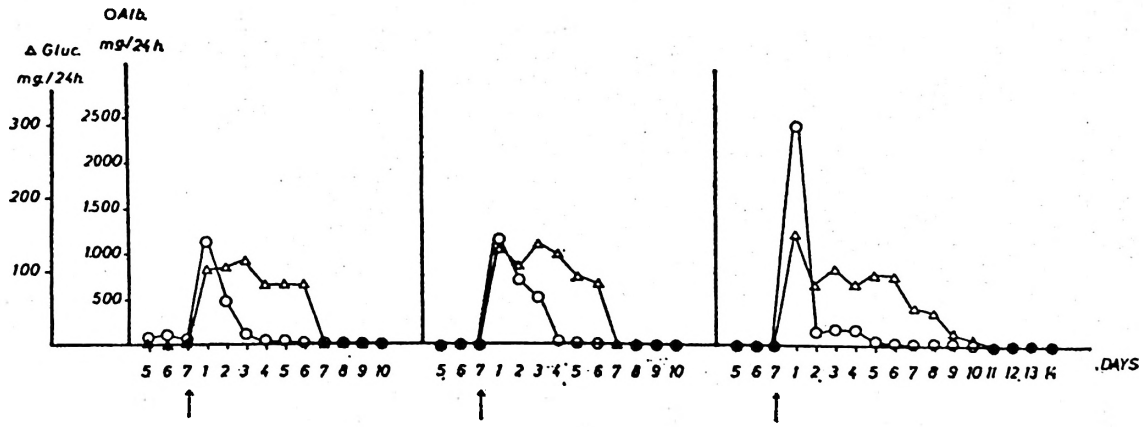


Fig. 1. *Curves of proteinuria* (○) *and glucosuria* (△) *in the series of rabbits intoxicated with 10, 13 and 15 ml/kg of glycerol solution respectively, from left to right.*

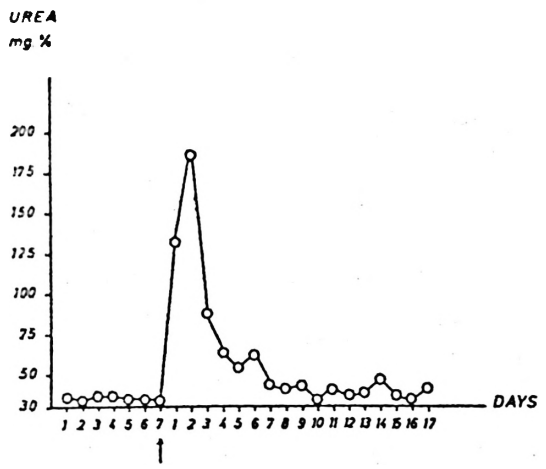


Fig. 2. *Curves (8 animals) of blood urea changes after intoxication with 13 ml/kg of glycerol solution.*

after 24 hours (+++); it is intense after 48 hours (++) and falls afterwards to a slightly positive reaction, persisting 3 to 6 days more.

*Changes of blood urea, pH, $pCO_2$ standard $CO_3H^-$ and plasma and urine ionogram.* Changes in blood urea have been examined in the animals intoxicated with 13 ml/kg. As it is shown in figure 2, after intoxication a very big increase appears in blood urea, with a peak maximum at 48 hours, which slowly goes down, arriving at initial values around two weeks. The changes in plasma und urine ionograms have been studied also in the lot of animals intoxicated with 13 ml/kg. As well in the animals dying as in those surviving, there is a most pronounced diminution of the urinary elimination of Na, K and Cl, diminution which attains its maximum (fig. 3) 48 hours post intoxicating, recuperating initial values slowly afterwards. Plasma K does not change in surviving animals and increase clearly in those which die. Plasma Na decreases in surviving animals during nearly 7 days, and is not affected in those which die. In

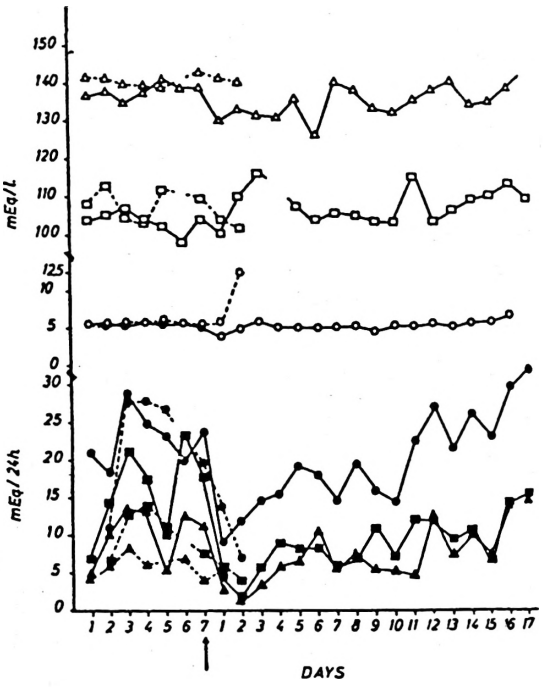


Fig. 3. *Curves of Cl, Na and K in the rabbits intoxicated with 13 ml/kg of glycerol, in three animals which die (---) and three other surviving (—).*
□ = plasma Cl, ■ = urine Cl, △ = plasma Na, ▲ = urine Na, ○ = plasma K, and ● = urine K. Two animals dead before 14 hours are not included.

plasma Cl the only appreciable change is an increase, for 3 days, in surviving animals.

In what concerns the plasma acid base equilibrium, a clear difference is seen between the animals which die and those surviving (fig. 4). In the former, an intense decrease of *p*H, $pCO_2$ and standard $CO_3H^-$ is observed. On the contrary, in the animals which do not die, *p*H increases slightly during the first 48 hours after intoxicating, but afterwards clearly during 6 days. $pCO_2$ diminishes clearly after 24 hours, raising slowly afterwards until reaching normal values. Standard $CO_3H^-$ does not change during the first 48 hours post intoxication, increasing afterwards during 6 to 7 days.

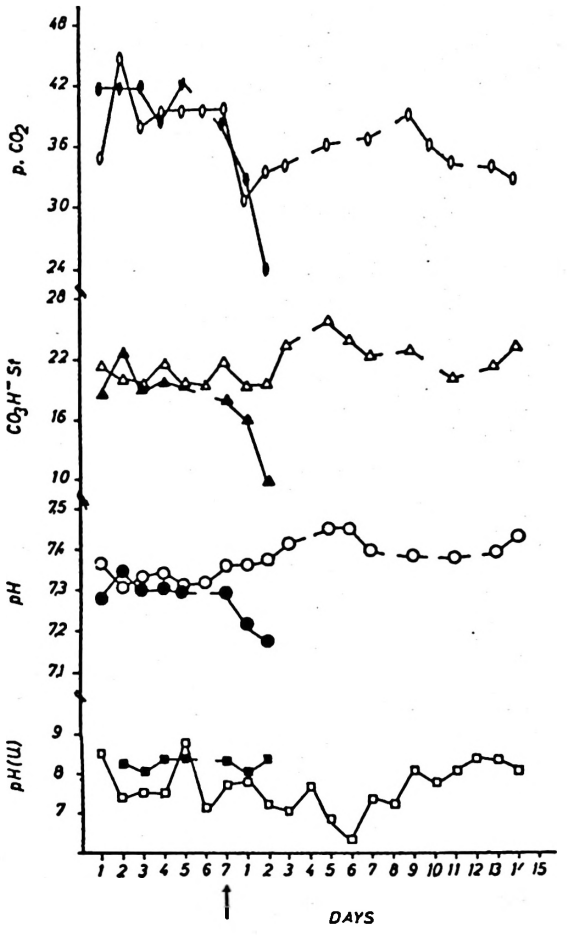


Fig. 4. *Curves of plasma $pCO_2$* (◇, ◆), pH (○, ●) *and standard $CO_3H^-$* (△, ▲) *in the rabbits intoxicated with 13 ml/kg of glycerol, in these animals which die (black) or survive (white).*
Mean curves of urine *p*H (□, ■), in the same animals.

*Histological lesions.* The predominant renal lesion is a tubular degeneration of different degrees, which generally affects the proximal tubule and appears in some cases in form of big drops, homogenous and confluent, of a material soluble in fat solvents. In the animals dead by intoxication, there is a tendency of calcium precipitate formation in the tubules, demonstrable by means of Von Kossa's staining. In what concerns the other organs we emphasize, because of their frequency, broncopneumonical lesions or pulmonary congestion, which may be produced by ethereal anesthesia (a synthesis of the degree of intensity and appearance of these lesions in both organs is shown in table II).

In the liver, moderate lesions of necrosis or degeneration of the hepatocyte are observed, predominating in the animals dead by intoxication, comparatively to those killed 14 or more days afterwards. No lesions are detected in other organs.

## Discussion

Histological studies were undertaken in the animals dead of intoxication as well as in those surviving, killed 14 to 17 days post intoxication; the data of the urine sediment and the biochemical ones on blood urea, proteinuria and glucosuria indicate that the top of lesional effect takes place between 24 hours and 6 to 7 days post intoxication. The histological data show that the lesions are localized chiefly in the proximal tubule, according to the data already existing in the rat (3, 4, 9, 10) and that in the animals killed after 14 days, they had almost completely disappeared. It is also intersting to point out that in the animals dead by intoxication, calcium precipitates are observed, like those described in human intoxication by xylitol or ethilenglicol (6, 8). No data on circulatory dynamics have been obtained for discerning if an ischemical phase is produced with subsequent diminution of the glomerula filtrate, as shown in the rat (12); considering the curves of diuresis post intoxication, it does not seem that oliguria is a predominant phenomenon post intoxication in the rabbit, at least if the animal has not been first submitted to water deprivation.

Histological and biochemical data do coincide, though substances reabsorbed by the proximal tubule, through a mechanism of maximal transport, like glucose,

Table II. *Histological lesions in kidney and lung of rabbits intoxicated with 10, 13 and 15 ml/kg of glycerol solution.*

| Rabbit | Days post Intoxi-cation* | KIDNEY | | | | | LUNG | |
|---|---|---|---|---|---|---|---|---|
| | | Glomerular Lesions | Tubular Degene-ration | Ca Precipi-tates | Cylin-ders | Infil-trates | Broncho-pneumonia | Con-gestion |
| 10 ml/kg | | | | | | | | |
| G-1 | 14 k | — | + | — | — | + | — | + |
| G-2 | 14 k | — | + | — | — | + | — | ++ |
| G-3 | 14 k | — | — | — | — | — | — | — |
| G-4 | 14 k | Congestive | Hydropic | — | — | — | — | — |
| G-5 | 14 k | — | — | — | — | — | — | ++ |
| G-6 | 14 k | — | — | — | — | — | + | — |
| G-7 | 14 k | — | ++ | — | — | — | — | — |
| G-8 | 14 k | Capsular Edema | — | ++ | — | — | + | — |
| G-9 | 14 k | — | + | — | — | + | Emphysema | + |
| 13 ml/kg | | | | | | | | |
| G-20 | 8 h d | — | +++ | — | ++ | ++ | +++ | — |
| G-29 | 8 h d | — | +++ | — | — | ++ | — | — |
| G-30 | 1 d | Edema Sclerosis | ++ | — | — | — | ++ | — |
| G-25 | 2 d | — | + | + | ++ | — | — | + |
| G-19 | 16 d | — | — | — | + | + | +++ | — |
| G-21 | 16 d | — | — | — | — | + | — | + |
| G-28 | 17 d | — | — | — | — | — | — | — |
| 15 ml/kg | | | | | | | | |
| G-11 | 1 d | — | ++ | — | + | + | — | ± |
| G-18 | 1 d | Cellular Increase | +++ | + | ++ | — | +++ | — |
| G-13 | 2 d | — | ++ | — | + | + | + | + |
| G-14 | 2 d | — | +++ | + | + | ++ | ++ | — |
| G-15 | 2 d | Edema Cellular | ++ | — | + | — | ++ | — |
| G-16 | 4 d | Increase | +++ | + | + | — | +++ | — |
| G-10 | 12 d | — | +++ | + | ++ | + | + | — |
| G-12 | 14 d | — | — | ++ | — | — | — | ± |
| G-17 | 14 d | Flocculus slightly enlarged | ++ | — | + | + | — | + |

* k = killed; d = dead; h = hour.

proteins and aminoacids, appear in the urine after 24 hours of intoxication, and glucose (from which we are better aware that its reabsorption needs energy [ATP]) is eliminated during 6 or 7 days. These data of glucosuria coincide with the clinical findings in cases of acute renal insufficiency.

A most evident fact is the considerable decrease in urinary elimination of Na, K and Cl, after 24 hours to intoxication in all animals. In those which die a little time after intoxication, plasma K increases clearly, and *p*H, $pCO_2$ and standard $CO_3H^-$ fall a great many. In those surviving Na decreases during a period of about 7 days, *p*H increases, especially after 48 hours and remains elevated about

6 days, $pCO_2$ decreases clearly after 24 hours, coming back to normal volume in a slow and progressive way, and the most surprising result is the increase in *p*H and of standard $CO_3H^-$ after 48 hours of intoxication. The interpretation of these data seems a little complex. The cause of the rapid death may be attributed to acidosis and increase of K; the great decrease of $pCO_2$ and standard $CO_3H^-$ indicates that this acidosis must be a metabolical one. Nevertheless, in $pCO_2$ decrease a compensating hyperventilation may play some role. In the surviving animals this acidosis and K increase are nof found and, after 48 hours, an increase, lasting several days, in blood *p*H and standard $CO_3H^-$ is found. This increase in standard $CO_3H^-$ is a strong argument in favor of a metabolic alcalosis, compensatory or not, but of renal origin. To our knowledge, this type of alcalosis has not been described.

There are demonstrative data in the hepatic cell, that glycerol store up in the endoplasmatic reticulum and afterwards in Golgi's apparatus (11), producing a great increase of alfa glycerophosphate, what induces a pronounced shortage of adenine nucleotides (13). This suggests that glycerol accumulation in the proximal tubule cells may produce a trouble of active reabsorption mechanisms needing ATP. On the other hand, the reabsorption mechanisms through pinocytosis phenomena may also be disturbed by haemoglobinuria, which reaches its highest intensity 24 and 48 hours post intoxication. It has been demonstrated, as well in clinical (2) as in experimental (1) haemoglobinuria, that the existing tubular lesions, similar to hialine degeneration, are phagosomes saturated with reabsorbed protein. It would be interesting to carry on further studies of this experimental pattern in the rabbit, intoxicating the animals without ethereal anesthesia, in order to eliminate possible masking factors.

## Resumen

Se estudia la aparición de una insuficiencia renal aguda en el conejo, postinyección de glicerol (10, 13 ó 15 ml/kg de una solución al 50 %). Veinticuatro horas después de la intoxicación y sobre todo durante unos 10 días, se encuentran cilindros, hematíes y células renales en el sedimento urinario. La proteinuria se observa a las 24 horas y casi ha desaparecido después de 72 horas. La glucosuria persiste de 24 horas a 6 dias. La hemoglobinuria es intensa después de 24 y 48 horas y persiste ligeramente durante 6 días. La eliminación de Na, K y Cl en la orina disminuye francamente en todos los animales. El K plasmático aumenta en los animales que mueren y no cambia en los que sobreviven. El Na plasmático no cambia en los que mueren y desciende en los que sobreviven. El *p*H, $pCO_2$ y $CO_3H^-$ descienden fuertemente en los animales que mueren. En los que no mueren, el $pCO_2$ desciende francamente después de 24 horas, para aumentar seguidamente poco a poco hasta alcanzar valores normales. El *p*H aumenta ligeramente durante las primeras 24 horas, y después claramente durante unos 6 días. El $CO_3H^-$ estándar no cambia durante las primeras 48 horas, para aumentar seguidamente durante 6 a 7 días. Desde el punto de vista histológico, la lesión principal consiste en una degeneración vacuolar del túbulo proximal. Se discuten los posibles mecanismos de estas alteraciones.

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