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CHANGES IN ELECTROLYTE BALANCE IN THE GASTROINTESTINAL SYNDROME

by

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Radiation damage leading to 'intestinal death' typically manifests itself in the so-called 'gastrointestinal syndrome', with diarrhoea, vomiting, anorexia, loss of weight, dehydration, and salt deficiency. The dosage range for intestinal death in laboratory animals is 1 000 to 12 000 r whole body irradiation (1, 20, 23); the survival time is 3 to 5 days, independent of the dose, hence the term '3 to 5-day-effect' (1, 9, 22). Underlying the pathologic process is radiation-induced damage to the intestinal mucous membrane brought about by both direct and indirect mechanisms. The direct radiation effect has been known since the days of Krause & Ziegler (16), and above all from the work of Regaud, Nogier & Lacassagne, published in 1912. It is evidenced morphologically by epithelial denudation of the intestinal mucosa which, approaching lymphatic tissue in its histologic structure, is highly sensitive to irradiation.

Evidence of acute erosive enterocolitis and lymphocytic infiltration is present in addition to the epithelial destruction of the crypts of Lieberkühn, oedema of the intestinal wall, and deeply penetrating ulcerative epithelial defects. Quaster, Lanzl, Keller & Osborne (21) in their publication 'acute intestinal radiation death' reported that irradiation with doses of more than 1 000 r of any major portion of the intestine alone produced lethal reactions in the rat. Additional evidence of the dominating role of the intestinal mucosa has been

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produced by shielding various lengths of the intestine (19), irradiating or extirpating isolated segments (25), and by experimental fluid and electrolyte therapy (4). It may thus be stated that the small intestine is the part of the digestive tube most sensitive to radiation, its radiosensitivity being about that of the skin. It is therefore obvious, and has been proved by experimental evidence, that radiation damage involves the intestine in manifold functional disorders, e.g. in the synthesis of deoxyribonucleic acid and ribonucleic acid. The barrier between the intestinal lumen and interior of the body breaks down and the intestinal absorption is reduced, as has been shown by BUCHWALD (5), BARRON et coll. (3), and MEAD et coll. (18) with glucose. QUASTLER (21) reached the conclusion that the immediate cause of intestinal death was the action of proteolytic enzymes and the loss of electrolytes and body fluid. The importance of the loss of electrolytes across the intestinal wall is clearly elucidated in the experimental work by e.g. BRECHER & CRONKITE (4), CASTER & ARMSTRONG (6), CONARD (7). JACKSON et coll. (13, 14) reported that sodium, in an amount lethal to the organism, is lost through the radiation-damaged intestine, and they suggested that this is because of the sodium being constantly secreted in the bile and not reabsorbed by the intestine. CURRAN et coll. (8), using radioisotopic methods, studied the absorption process in isolated intestinal loops of the rat, irradiated with 2 500 to 3 000 r, and found that 13 hours after irradiation the active absorption of both sodium and water ceased. GOODNER et coll. (10), studying in vivo absorption of ^{22}Na and H^3OH from the intestinal tract of the rat, reported that an absolute efflux of sodium and water from the blood of the lumen of the intestine occurred 48 hours after irradiation with 650 or 2 000 r.

The process responsible for the lethal loss of electrolytes that leads to 'intestinal death' is still obscure. Differences in results may be due to variations in experimental methods. LUSHBAUGH et coll. (17), using the radioisotopic tracer technique, determined the post-irradiation changes in the total body amounts of sodium and potassium in rats given whole body doses of 1 000 to 8 000 r. They found no excessive total body losses of these substances, and suggested that intestinal death might be due to local changes in body electrolytes and water, without alteration in their total body content.

The present experiments were designed to determine the quantitative changes that might occur in the body distribution of electrolytes, particularly in that of intracellular and extracellular sodium.

Material and Methods. Male Wistar-strain rats, maintained on daily rations of known composition and weighing 200 to 250 g, were deprived of food and water 18 hours before the beginning of the experiments; a number were marked to serve as controls.

Whole body irradiation was delivered to the experimental animals by a Siemens Stabilipan apparatus at a rate of 65 to 75 r/min, the total dose being

1 200 r, half-value layer 1 mm Cu. The actual dose rate measured in air was controlled before each irradiation by means of a Siemens Universal dosemeter. The animals to be irradiated were placed in a plexite box.

About 72 hours after irradiation, and following ligation of the two renal hila performed 30 minutes earlier, the experimental animals were injected intravenously with 8 to 10 μC radioactive sodium. The non-irradiated control rats were subjected to the same procedures at the same times. Data in the literature (11, 12, 15) having indicated that ^{24}Na takes from 3 to 6 hours to be uniformly distributed in the rat, all animals were bled to death 4 hours after the isotope injection by cardiac puncture. Each animal had 30 min previously been given 15 mg of inulin intravenously.

The activity of the ^{24}Na was determined in 1 ml of serum, the amount of ^{23}Na in 0.2 ml of serum diluted 1 : 20, and the inulin content in 0.5 ml of serum.

The sodium isotope activity of the tissues of the major organs (brain, heart, spleen, lung, kidney, small intestine), and of the femoral bone and muscle tissues, was measured in extracts obtained by decocting a 0.5 to 1 g specimen of the respective tissues together with 1 ml of a 20 % solution of sodium hydrate. The total sodium content was determined in decoctions of such tissue specimens in 16 N of citric acid, filtered and diluted 1 : 20.

Geiger-Müller and sodium iodide scintillation counters were used for measurement of the activity in serum and in the tissue decoctions.

The serum inulin content and inulin space were determined colorimetrically with diphenylamine by means of an 'Uvifot' photometer after removal of protein and sugar from the serum (26).

The total exchangeable sodium, i.e. that part of the whole body sodium in which the radioactive sodium spreads uniformly, was obtained from the following formula:

$$\frac{\text{activity of } ^{24}\text{Na administered}}{\dfrac{\text{serum } ^{24}\text{Na activity}}{\text{serum } ^{24}\text{Na content}}}$$

and expressed in μC Eq/100 g.

The exchangeable sodium content of the extracellular space was calculated from the inulin space (15). The latter was considered equivalent to the former, but this was without influence on the evaluation of our results since, at 16.4 $\pm$ 1.36 %, the inulin space had been found normal in these experiments and in accord with the latest data in the literature.

The exchangeable sodium content of the individual organ tissues, and of the bone and muscle tissues, was obtained from the expression

$$\frac{^{24}\text{Na content of 1 g tissue}}{\text{serum specific activity}}$$

and given as Eq per 1 g of tissue.

Table 1

Sodium contents in rats 76 hours after whole-body irradiation of 1 200 r

	Unit	Control n		Irradiated n		p
Exchangeable Na of E.C.S.	$\frac{\mu Eq}{100\ g}$	12	2 681 ± 113	7	1 187 ± 192	$\ll 0.01$
Total exchangeable Na	$\frac{\mu Eq}{100\ g}$	13	5 035 ± 282	9	4 380 ± 399	< 0.01
^{24}Na space	$\frac{ml}{100\ g}$	13	32.23 ± 3.24	9	31.98 ± 1.59	> 0.20
Inulin space	$\frac{ml}{100\ g}$	12	16.4 ± 1.36	7	8.9 ± 1.05	$\ll 0.01$
Serum Na	$\frac{mEq}{lit.}$	12	149 ± 4.12	8	135 ± 6.67	

n: number of determination

Results

The total exchangeable sodium of the non-irradiated control rats was 5 035 μEq/100 g, as seen from Table 1; the value is in agreement with that found by other authors (12). This is less than the total body sodium, a considerable proportion of which is to be found in the bones. Radioactive sodium is known to take 12 hours to mix intimately with the sodium in the osseous system, whereas the time commonly allowed to elapse between introduction of the isotope and blood sampling is 4 to 5 hours (11, 12). In the present experiments, more than half of the total exchangeable sodium, or exactly 2 681 μEq/100 g, was made up of exchangeable sodium of the extracellular space. This value comes near to the total sodium of the extracellular space because the equilibrium between ^{24}Na and ^{23}Na is soon restored in the extracellular fluid.

It is further seen in Table 1 that 76 hours after whole body irradiation with 1 200 r the extracellular sodium content decreased considerably, by 1 490 μEq/100 g. Only a part of this left the organism, for the total exchangeable sodium decreased only by 655 μEq/100 g. The sodium isotope activity of the small intestine, which is included in the total exchangeable sodium, was found to have risen above the serum activity values.

The amount of exchangeable sodium present in the intestinal contents will be determined in separate experiments, and compared with the present results in a future communication, as soon as certain difficulties in techniques have been overcome.

The changes in exchangeable sodium of the tissues of the individual organs, and in bone and muscle, observed at 76 hours after whole body irradiation with 1 200 r, are summarized in Table 2.

Only muscle tissue shows a significant decrease ($p < 0.01$) and bone tissue a significant increase ($p < 0.02$) in exchangeable Na.

Table 2

Variations in the exchangeable sodium content of some tissues of major organs of rat 76 hours after 1 200 r of whole-body irradiation

Tissue	Control rats			Irradiated rats			Difference
	n	μEq/1g	S. D.	n	μEq/1 g	S. D.	μEq/1 g
Brain	14	34.13	± 2.24	10	35.94	± 7.80	+ 1.81
Lung	14	49.80	± 4.72	10	44.00	± 10.73	+ 5.80
Liver	14	23.90	± 2.11	10	19.95	± 5.50	— 3.95
Spleen	14	22.71	± 3.41	9	24.40	± 3.74	+ 1.69
Small intestine	14	30.20	± 3.51	10	35.20	± 12.53	+ 5.00
Heart	14	19.01	± 2.65	10	21.87	± 3.31	+ 2.86
Muscle	14	20.31	± 3.72	10	12.70	± 3.15	— 7.61
Bone	13	65.00	± 4.44	10	76.93	± 10.08	+ 11.93

n: number of determinations

By the terms of the expression stated under 'Methods', the exchangeable sodium in the individual organ — which the authors considered equivalent to the total sodium content — is obtained if the Na-isotope mixes intimately with the stable isotope content of the organism. Four hours after administration of the isotope, the individual organs were saturated to 90 % only (calculated from the expression $\frac{\text{tissue specific activity}}{\text{serum specific activity}} \times 100$); the exchangeable sodium in the organs was consequently found to be less than the total sodium. The degree of saturation was the same for all the tissues studied excepting those of the bone and brain. Because of the wide scatter, the flame-photometrically measured total sodium contents of the organs were utilized solely for determining saturation degrees.

Discussion

The changes in electrolyte balance, produced in the intracellular and extracellular spaces by loss of sodium and body fluid, in 'intestinal death' induced by irradiation with 1 200 r, has been investigated.

It was found that the exchangeable sodium content of muscle tissue had decreased while that of bone tissue had increased. These findings agree with those of CASTER & ARMSTRONG (6) with 700 r. This, referred to the whole body, means that against a sodium loss of about 320 μEq/100 g in muscle tissue may be set a sodium uptake of about 420 μEq/100 g by bone tissue. The changes in the intracellular sodium of the organism could not have been substantial in relation to the sodium loss through the intestine in the terminal stage since no noteworthy changes occurred in the exchangeable sodium contents of the organ tissues (Table 2).

The significant decrease in extracellular sodium, which in the 76th hour amounted to 1 490 Eq/100 g, is remarkable. JACKSON & ENTENMAN (14) stated that rats irradiated with 1 500 r lose 1.50 mEq of sodium through the

intestinal tract and that, following intraperitoneal dialysis, a loss of 1.40 to 1.50 mEq results in death within 24 hours. It would appear, on the strength of the agreement between the data and the known impairment of the activity of the intestinal mucosa, that loss of intestinal sodium occurs chiefly at the expense of extracellular sodium.

The total exchangeable sodium was found to have decreased by 650 Eq, but from the point of view of the whole organism this was of little consequence. The lethal change in the electrolyte balance was caused by the amount of sodium (1 490 μEq) that had passed into the gastrointestinal tract, from which only a part had left the organism. Comparison of the extracellular spaces, as determined by Na-isotope and inulin methods, verified the occurrence of this passage: the Na space exhibited no essential change because it included the fluid stagnant in the gastrointestinal tract amounting to 10 % of the total body fluid (MOSER, ref. 20). The inulin space, which represents the effective extracellular space of the organism, likewise decreased, i.e. from 16.4 % to 8.9 % (Table 1). The foregoing explains why the decrease in extracellular sodium, which is always concomitant with contraction of this space, failed to alter the serum sodium concentration to any marked extent.

It should be pointed out in conclusion that a sound electrolyte balance, influenced as it is by endocrine, circulatory and metabolic factors, depends on the integrity of the system governing and controlling it.

SUMMARY

The changes in electrolyte balance immediately preceding 'gastrointestinal death' were studied with Na-isotope and inulin methods in rats irradiated with 1 200 r. The terminal stage of the gastro-intestinal syndrome was found to be characterized by passage of sodium from the extracellular space into the damaged gastro-intestinal tract.

ZUSAMMENFASSUNG

Veränderungen der elektrolytischen Bilanz kurz vor 'gastro-intestinalem Tod' wurden mit Hilfe von Sodiumisotopen und Inulinmethoden an Ratten studiert, die mit 1 200 r bestrahlt worden waren. Das Endstadium des 'gastro-intestinalen' Syndromes im Versuchstier ist charakterisiert durch die Passage von Sodium aus dem extrazellulären Raum in den beschädigten Gastro-Intestinalkanal.

RÉSUMÉ

Les perturbations de l'équilibre électrolytique précédant immédiatement la 'mort gastro-intestinale' ont été étudiées par les méthodes au sodium radioactif et à l'inuline sur des rats irradiés par 1 200 r. Le stade terminal du syndrome gastro-intestinal est caractérisé par le passage du sodium de l'espace extra-cellulaire vers le tube digestif gravement lésé.

REFERENCES

1. BACQ Z. M., and ALEXANDER P.: Fundamentals of radiobiology. 2nd edition. Pergamon Press, London 1961.
2. BALINT P.: Klinikai Laboratoriumi Diagnosztika. 3. kiad, Medicina, Budapest 1962.

3. Barron E. S. G., Wolkowitz W., and Muntz J. A.: The effect of X-rays on the metabolism of small intestine and its permeability to glucose. Atomic Energy Comission Document, MDDC-1241 (1947).
4. Brecher G., and Cronkite E. P.: cited by Quastler.
5. Buchwald K. W.: Influence of x-ray lesions of intestinal mucosa on absorption of glucose and other sugars. J. exp. Med. 53 (1931), 827.
6. Caster W. C., and Armstrong W. D.: Elektrolyte metabolism after total-body x-irradiation. Radiat. Res. 5 (1956), 189.
7. Conard R. A.: Some effect of ionizing radiation on the physiology of the gastrointestinal tract: a review. Radiat. Res. 5 (1956), 167.
8. Curran F. P., Webster W. E., and Hovsepian J. A.: The effect of x-irradiation on sodium and water transport in the rat ileum. Radiat. Res. 13 (1960), 369.
9. Gerber G.: Untersuchungen über den Einfluss der Nebenniere nach Ganzkörperbestrahlung von Mäusen mit Dosen im Konstanzbereich („3,5-Tage Effekt"). Strahlentherapie 110 (1959), 510.
10. Goodner C. J., Moor Jr T. E., Bowers Z. J., and Armstrong W. D.: Effects of acute whole body x-irradiation on the absorption and distribution of Na^{22} and H^3OH from the gastrointestinal tract of fasted rat. Amer. J. Physiol. 183 (1955), 475.
11. Green D. M., Reynolds T. B., and Girard R. J.: Determination of total tissue sodium concentrations by use of radiosodium. Circulat. Res. 3 (1955), 330.
12. Hársing L., Dubecz E., Kövér Gy., and Nágy S.: Hypothermia hatása a kicserélhetö Na-mennyiségre. Kisérletes orvostudomány 13 (1961), 373.
13. Jackson K. C., Rhodes R. L., and Entenman C.: Electrolyte metabolism after total-body x-irradiation. Radiat. Res. 8 (1958), 361.
14. Entenman C.: The role of bile secretion in the gastrointestinal radiation syndrome. Radiat. Res. 10 (1957), 67.
15. Kovács A.: Kisérletes Orvostudomány vizsgáló módszerei. Akadémiai Kiadó, Budapest 1954.
16. Krause P., und Ziegler K.: Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf tierisches Gewebe. Fortschr. Röntgenstr. 10 (1906), 126.
17. Lushbaugh C. C., Sutton J., and Richmond C. L.: The question of electrolyte loss in the intestinal death syndrome of radiation damage. Radiat. Res. 13 (1960), 814.
18. Mead J. F., Decker A., and Bennett L. R.: Effect of x-irradiation upon fat absorption in mouse. J. Nutr. 43 (1951), 485.
19. Osborne J. W.: Prevention of intestinal radiation death by removal of the irradiated intestine. Radiat. Res. 4 (1956), 541.
20. Quastler H.: The nature of intestinal radiation death. Radiat. Res. 4 (1956), 303.
21. — Lanzl E. F., Keller M. E., and Osborne J. W.: Acute intestinal radiation death; studies on roentgen death in mice. Amer. J. Physiol. 164 (1951), 547.
22. Rajewsky B.: Strahlendosis und Strahlenwirkung. 2. Auflage. G. Thieme Verlag, Stuttgart 1956.
23. Regaud C., Nogier T., et Lacassagne W.: Sur les effects redoutables des irradiations étendues de l'abdomen et sur les lesions du tube digéstif détérminées par les rayons de roentgen. Arch. Élect. méd. 21 (1912), 331.
24. Soberman R. J., Keating R. P., and Maxwell R. D.: Effect of acute whole-body x-irradiation upon water and electrolyte balance. Amer. J. Physiol. 164 (1951), 450.
25. Takamaya H., Nishioka K., and Sunada T.: Observation on the effect of protection of the intestine on the lethality produced by total body x-irradiation in rats. Med. J. Osaka Univ. 10 (1960) Nos 3—4.
26. Zsebök Z., und Petrányi Jr, Gy.: Experimentelle Untersuchungen über die Veränderung des extracellulären Raumes beim Strahlensyndrom. Acta med. Acad. Sci. Hung. 19 (1963), 137.