Changes in arterial and mixed venous pH, PCO_2 and PO_2 following DNP injections

Total dose of DNP (mg/kg)	Arterial blood			Mixed venous blood		
	pH	pCO_2	pO_2	pH	pCO_2	pO_2
0 (control)	7.44 ± 0.05	26.5 ± 4.2	81.9 ± 6.9	7.40 ± 0.07	33.7 ± 6.4	42.0 ± 4.7
5	7.48 ± 0.04	24.3 ± 2.8	83.1 ± 10.7	7.43 ± 0.04	30.0 ± 4.5	37.5 ± 6.0
10	7.50 ± 0.05	21.8 ± 2.9*	80.9 ± 9.0	7.45 ± 0.03*	28.6 ± 4.8*	31.8 ± 5.0***
15	7.52 ± 0.06*	21.0 ± 1.6*	80.9 ± 4.5	7.44 ± 0.05	28.3 ± 3.0*	27.7 ± 4.2***
20	7.51 ± 0.03**	19.6 ± 2.3***	79.4 ± 14.4	7.44 ± 0.05	27.0 ± 4.4*	26.2 ± 4.5***

Statistical significance of changes from control by unpaired Student's t-test: *$0.90 < p < 0.95$; **$0.95 < p < 0.99$; *** $p > 0.99$.

The effect of the drug injections was found to be cummulative. Results are shown in the table.

Lung ventilation was not measured quantitatively, but a drastic rise in both tidal volume and breathing frequency was evident in every case. Rises in body (rectal) temperature were minimized by tracheotomy and never exceeded 1–1.5 °C (means: 37 °C ± 0.8 SD at the beginning, and 38.2 °C ± 0.6 SD at the end of experiments). Mean arterial blood pressure did not change.

Changes, observed in arterial and mixed venous blood composition suggest that in the case of DNP-induced ventilatory rise neither CO_2 nor pH is a respiratory stimulus. Gradual decrease in arterial PCO_2 accompanied by respiratory alkalosis were observed. Mixed venous PCO_2 and pH changes followed that of arterial PCO_2 and pH. Arterial and mixed venous hypocapnia could be explained by a shift in respiratory quotient; it is also possible that increase in cardiac output, which follows DNP infusions[6], together with increased lung ventilation, would favour CO_2 diffusion in lungs, because of substantial difference in diffusibility of CO_2 and O_2 across alveolar walls[7].

If perhaps CO_2 is more efficiently removed from blood than oxygen is dissolved, the possible mechanism might be suggested, which drives respiration to assure adequate supply of oxygen to the tissues rather than to stabilize arterial PCO_2. Therefore, in the condition of DNP-induced hypermetabolism, the role of carbon dioxide in the respiratory control would be of no essential importance. Both increase in cardiac output and lung ventilation were recently shown by Liang and Hood[8] to be related to mucsle afferents stimulation, resulting from DNP action. On the basis of those results it seems likely that muscle receptors play a substantial role in the ventilatory response to DNP. It is, however, uncertain whether increase in lung ventilation is directly related to the metabolic, i.e. chemical stimulus, or results from an increase in cardiac output[9]. If there is also an additional stimulation of ventilation, related to blood gas content, decrease in venous oxygen would be the only stimulus.

6 C. S. Liang and W. B. Hood, J. clin. Invest. *52*, 2283 (1973).
7 J. H. Comroe, in: Physiology of Respiration. Chicago, p. 140. Year Book Medical Publishers, Inc. 1965.
8 C. S. Liang and W. B. Hood, Circulation Res. *38*, 209 (1976).
9 K. Wasserman, B. J. Whipp and J. Castagna, J. appl. Physiol. *36*, 457 (1974).

Peritoneal dialysis in small laboratory animals[1]

P. G. Lankisch[2,3], H. Koop, K. Winckler, E. Quellhorst and H. Schmidt

Division of Gastroenterology and Metabolic Diseases, Department of Medicine, University of Göttingen and Nephrological Centre Niedersachsen, Hann.–Münden (Federal Republic of Germany), 1 October 1976

Summary. Healthy rats and guinea-pigs were treated with a simple method of continuous peritoneal dialysis for 12, 24 and 48 h. Increasing with time, both animal species developed severe hypoproteinemia and hemoconcentration due to protein loss into the dialyzate fluid. These changes were associated with a high mortality rate, when Sterofundin® was used for dialysis. Therefore, protein loss should be substituted and the type of dialyzate must be considered in experimental long-term dialysis using these small laboratory animals.

Peritoneal dialysis is widely used in the treatment of renal and non-renal diseases in man. As animal experiments were reported only in dogs[4,5] and rabbits[6] requiring difficult operative procedures, a simple experimental model was developed for small laboratory animals[7]. Our experiments show that prolonged peritoneal dialysis in these animals is associated with considerable protein loss requiring substitution in long-term experiments.

Material and methods. Peritoneal dialysis was carried out in 101 male Wistar rats (180–250 g) and 25 guinea-pigs (240–320 g). Operative procedure is described else-

1 Supported by Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, La 369/1.
2 Reprints requests should be addressed to P. G. Lankisch, Medizinische Universitäts Klinik, Humboldtallee 1, D–3400 Göttingen.
3 Acknowledgment. Our thanks are due to Jutta Otto and Ulrich Oberdieck for expert technical assistance.
4 R. E. Rodgers and L. C. Carey, Am. J. Surg. *111*, 792 (1966).
5 E. F. Rosato, J. C. Oram-Smith, W. F. Mullis and F. E. Rosato, Ann. Surg. *175*, 384 (1972).
6 E. Dziuk and M. Siekierzynki, Acta physiol. pol. *24*, 465 (1973).
7 P. G. Lankisch, H. Koop, K. Winckler, E. Quellhorst and H. Schmidt, Clin. Nephr. *4*, 251 (1975).

Table 1. Serum protein and hematocrit of rats, guinea-pigs and patients with chronic renal insufficiency after different intervals of peritoneal dialysis with Sterofundin® and Peritosteril HK®

	Controls x ± SD (n)		Dialyzate fluid	Peritoneal dialysis 12 h x ± SD (n)	24 h x ± SD (n)	48 h x ± SD (n)
Guinea-pig	Serum protein (g/dl)	5.3 ± 0.3 (9)	Sterofundin®	4.3 ± 0.7** (7)	4.6 ± 0.5*** (5)	3.1 ± 0.2* (5)
	Hematocrit (%)	39.7 ± 2.6 (10)		40.9 ± 4.7 (7)	57.9 ± 6.9* (6)	61.6 ± 3.8* (4)
Rats	Serum protein (g/dl)	6.6 ± 0.5 (33)	Sterofundin®	4.6 ± 0.4* (15)	4.0 ± 0.4* (13)	3.5 ± 0.5* (7)
	Hematocrit (%)	43.1 ± 3.5 (15)		48.1 ± 4.3** (15)	42.0 ± 5.6 (14)	56.3 ± 7.0* (10)
	Serum protein (g/dl)	6.6 ± 0.5 (33)	Peritosteril HK®	–	5.7 ± 0.6* (12)	3.8 ± 0.6* (18)
	Hematocrit (%)	43.1 ± 3.5 (15)		–	48.0 ± 3.6** (11)	46.2 ± 7.0 (19)
Patients (12)	Serum protein (g/dl)	6.2 ± 0.9+	Peritosteril HK®	–	6.5 ± 1.0	–
	Hematocrit (%)	26.7 ± 4.0+		–	28.2 ± 4.0	–

+, before peritoneal dialysis; *, $p < 0.001$; **, $p < 0.005$; ***, $p < 0.01$ versus controls.

where[7]. Briefly, a polyethylene catheter is carefully inserted under ether anesthesia through a needle pushed s.c. from the neck into the lateral part of the abdominal cavity. A small silicone cuff placed beneath the peritoneum prevents withdrawal of the intra-abdominal part of the catheter which carries 6–8 lateral perforations.

Continuous peritoneal dialysis was performed with Sterofundin® (B. Braun, Melsungen, Federal Republic of Germany) and Peritosteril HK® (E. Fresenius KG, Bad Homburg v.d.H., Federal Republic of Germany) by injection of 15 ml at 30 min intervals up to 48 h. Sterofundin® contains Na^+ 140 mval/l, K^+ 4 mval/l, Ca^{++} 5 mval/l, Mg^{++} 2 mval/l, Cl^- 106 mval/l, Lactate 45 mval/l; pH 5.9, 315 mosm/l. Peritosteril HK® contains Na^+ 127 mval/l, K^+ 4 mval/l, Ca^{++} 2.6 mval/l, Mg^{++} 1 mval/l, Cl^- 99.6 mval/l, acetate 35 mval/l, sorbit 20 g/l; pH 6.8, 377 mosm/l. During peritoneal dialysis, the animals were alert and moved around freely in their cages.

For comparison with the animal experiments, serum protein and hematocrits were measured in 12 patients with chronic renal failure before and after peritoneal dialysis for 24 h. Serum protein was measured by the Biuret method[8] and hematocrits in heparinized capillary tubes. Serum electrophoresis was performed according to Kohn[9]. For statistical analysis the t-test was used.

Results. Using Sterofundin®, there was a recovery of the administered fluid of 99.2 ± 4.4% in guinea-pigs (n = 24) and of 95.0 ± 6.6% in rats (n = 68) after 12 h peritoneal dialysis. When Peritosteril HK® was applied the recovery was 103.7 ± 2.9% (n = 32).

During Sterofundin®-dialysis, there was a hemoconcentration up to 61.6% in guinea-pigs and 56.3% in rats after 48 h. Hemoconcentration was less when Peritosteril HK® was used (table 1). Serum protein levels decreased to 53–58% of controls after 48 h of peritoneal dialysis (table 1). Protein loss in the Sterofundin®-groups was obviously more severe than was reflected by the decrease in serum protein levels, since it was partially hidden by marked hemoconcentration. After 12 h, albumin was reduced by 50% and globulins by 18%, compared with normals. When Sterofundin® was used, mortality rate was 44.4% in guinea-pigs and 53.8% in rats after 48 h of peritoneal dialysis (table 2). No animal died during dialysis with Peritosteril HK® up to 48 h.

In contrast to guinea-pigs and rats, patients with chronic renal failure dialysed with Peritosteril HK® showed no changes of serum protein and hematocrit (table 1).

Discussion. Protein loss with the dialyzate fluid is known in peritoneal dialysis in man[10]. Yet, in patients with chronic renal failure we could not observe changes in serum protein values and hematocrit after 24 h of peritoneal dialysis. In contrast, rats and guinea-pigs developed severe hypoproteinemia during peritoneal dialysis for 24–48 h. Orientating estimations of protein content in the

8 T. E. Weichselbaum, Am. J. clin. Path. *10*, 40 (1946).
9 J. Kohn, Ärztl. Lab. *10*, 233 and 269 (1964).
10 R. A. Palmer, J. E. Newell, E. J. Gray and W. E. Quinton, New Engl. J. Med. *274*, 248 (1966).

Table 2. Mortality rate of rats and guinea-pigs during continuous peritoneal dialysis for 12, 24 and 48 h with Sterofundin® and Peritosteril HK®

Species	Dialyzate fluid	Peritoneal dialysis 12 h	24 h	48 h
Guinea-pigs	Sterofundin®	2/25 (8%)	3/17 (18%)	4/9 (44%)
Rats	Sterofundin®	1/69 (1%)	1/48 (2%)	14/26 (54%)
Rats	Peritosteril HK®	0/32 (–)	0/32 (–)	0/20 (–)

effluent dialyzate fluid show that this is mainly due to a high protein loss into the peritoneal cavity, which obviously cannot be compensated by de novo synthesis. The reason for the high protein leakage in these small laboratory animals is not known. It is suggestive that the high mortality rate after 48 h of dialysis with Sterofundin® may be connected with the hypoproteinemia. Yet, 48 h after dialysis with Peritosteril HK® no animals had died, although serum protein was reduced to a similar degree. Therefore the properties of the dialyzate fluid seem to be essential for survival. The composition of Sterofundin® and Peritosteril HK® differ in many respects including concentration of cations and types of anions as well as pH-values and osmolarity. At present it is a matter of speculation which parameters may be most important in regard to the mortality rate.

It can be concluded from our experiments that dialyzate fluids of the type of Peritosteril HK® should be used for continuous dialysis in experimental studies with rats and guinea-pigs, and that protein must be replaced during long-term treatment to avoid hypoproteinemia and hemoconcentration.

Under these circumstances, the method described is a simple, quick and cheap technique for peritoneal dialysis in small laboratory animals. It was successfully used in the treatment of experimental acute pancreatitis in more than 300 rats[11].

11 P. G. Lankisch, H. Koop, K. Winckler and H. Schmidt, Biol. Gastroenterol. *8*, 351 (1975).

Changes in gastro-intestinal serotonin content associated with fasting and satiation

G. Biggio, M. P. Piccardi, M. L. Porceddu and G. L. Gessa

Institute of Pharmacology, University of Cagliari, via Porcell 4, I–09100 Cagliari (Italy), 20 December 1976

Summary. Rats fasted for 24 h were fed for 3 h, after which time food was removed. Food intake decreased serotonin levels in the stomach and duodenum by 30 and 40%, respectively. These changes persisted for about 3 h. Food intake did not change tryptophan content in the stomach, while, in the duodenum, tryptophan level rose by 100% at the end of the feeding period and remained elevated for about 9 h.

High concentrations of serotonin (5-HT) are present in the gastro-intestinal tract of all vertebrates. This amine is mainly localized in the enterochromaffin cell system of the mucosa[1,2], although small concentrations have been found also in the intramural nervous system of the gastro-intestinal tract[3,4]. It has been suggested that 5-HT has a stimulating action on gastro-intestinal motility and, therefore, that this monoamine might function in the modulation of the peristaltic reflex[5–7]. Modifications in the level of this monoamine have been obtained with the use of diets free from, or lacking in, tryptophan[8,9], by modifying the normal intestinal flora[10,11], or by administering drugs acting on monoamine metabolism[12,13].

This report shows that gastro-intestinal 5-HT content undergoes changes associated with fasting and satiation.

Materials and methods. Experiments were carried out with male Wistar rats, initially weighing 150–180 g. The rats were housed 3 per cage in wire-bottom cages at a room temperature of 24 °C with reversed light-dark cycle: lights on from 22.00 to 10.00. They had access to water ad libitum but were trained to consume their normal food intake in a period of 3 h: food was presented ad libitum at 11.00 and removed at 14.00. Experiments were carried out after 3 weeks of training. At this time, each rat ate an average of 18.6 ± 0.5 g of the diet per day. Rats were fed

1 V. Erspamer, Pharmac. Rev. *6*, 425 (1954).
2 W. Feldberg and C. C. Toh, J. Physiol. *119*, 352 (1953).
3 M. D. Gershon, A. B. Drakontides and L. L. Ross, Science *149*, 197 (1965).
4 M. D. Gershon and L. L. Ross, J. Physiol. *186*, 477 (1966).
5 E. Buldring and A. Crema, Br. J. Pharmac. *13*, 444 (1958).
6 E. Buldring and R. C. Y. Lin, J. Physiol. *138*, 120 (1957).
7 B. J. Haverback and S. K. Wirtschaftler, Adv. Pharmac. *1*, 309 (1962).
8 D. J. Boullin, Psychopharmacologia *5*, 28 (1963).
9 E. M. Gal and P. A. Drewes, Proc. Soc. exp. Biol. (N. Y.) *110*, 368 (1962).
10 R. S. Stacey and T. J. Sullivan, J. Physiol. *137*, 63 (1957).
11 T. J. Sullivan, Br. J. Pharmac. *16*, 90 (1961).
12 K. Iwata and H. Okamoto, Experientia *29*, 988 (1973).
13 K. S. Kim and P. A. Shore, J. Pharmac. exp. Ther. *141*, 321 (1963).

Changes in gastro-intestinal serotonin and tryptophan levels associated with fasting and satiation

Duration of fasting time interval after last meal, h	Stomach Tryptophan (μg/g)	Serotonin μg/g)	Duodenum Tryptophan (μg/g)	Serotonin (μg/g)
24	7.35 ± 0.18	1.31 ± 0.18*	12.42 ± 0.24*	4.33 ± 0.12*
9	7.39 ± 0.29	1.25 ± 0.31*	18.21 ± 0.29*	4.74 ± 0.18*
3	7.41 ± 0.26	0.91 ± 0.24	24.23 ± 0.21	3.08 ± 0.15
0 (at the end of feeding period)	7.02 ± 0.15	0.86 ± 0.10	23.91 ± 0.07	2.97 ± 0.09

Each point is the average ± SE of 16 animals. Animals fasted 24 h were allowed to eat for 3 h after which time food was removed (zero time). The duration of fasting (h) reported indicates the intervals between food removal and death. * $p < 0.01$ compared to the value for 0 h of fasting.