of a local strain were used in this study. Selection and maintenance of the rats were as previously described[19]. The rats were housed individually in a wheelrunning apparatus to which was attached a small living cage. Exercising rats had free access between the cage and the drum. The mean voluntary wheelrunning activity of this group by the end of the experiment was 15.0±0.6 km/24 h. In the nonexercising group, the admittance to the drum was closed with a metal plate. The rats were fed the purified high sucrose formula diet A, previously reported[20], and water ad libitum. At the start of the experiment, the exercising and nonexercising groups were selected so as to provide equal mean levels of running activity. Blood samples were obtained after 3 months for determination of plasma cholesterol and triglyceride concentration, and for carrying out lipoprotein electrophoresis. Blood sampling and the analytical methods were as previously described[19,20].

Results and discussion. The concentration of plasma cholesterol was remarkably similar in exercising and nonexercising groups (figure 1). In contrast to this, the mean level of plasma triglycerides was reduced by more than 50% in the exercising group as compared with the nonexercising group. B.wts were not different in the 2 groups.

The percentage of pre-β and β-lipoproteins were quite similar in exercising and nonexercising rats (figure 2). The 'pre-α fraction', which consists of several diffuse bands between the β- and α-bands, was nearly as large as the α-fraction. There was a slightly increased percentage of 'pre-α' lipoproteins in the nonexercising group as compared with the exercising group. α-lipoprotein percentages were not significantly different in the 2 groups.

These results suggest that the level of voluntary physical activity in rats does not greatly influence the plasma lipoprotein distribution. Our observation is in contrast to the finding of Lopez-S et al.[18], who reported that α-lipoproteins increased and β-lipoproteins decreased in response to physical activity in young men. This effect was, however, small. Moreover, maximal exercise was performed. The observation that physical activity did not influence the cholesterol level is in agreement with previous studies[5-8], as is also the reduction by physical activity of plasma triglycerides concentration[2-4,7,9]. It should, however, be pointed out that in a similar study, in which the rats were fed a stock diet, no effect of voluntary physical activity on the plasma triglyceride level was observed[19]. Thus, the triglyceride response to physical activity seems to depend upon the diet used. It would appear that diet is more important than physical activity, both for the regulation of plasma lipid concentration and lipoprotein distribution.

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2 R.J. Barnard, R.L. Terjung and C.M. Tipton, Int. Z. angew. Physiol. *25*, 303 (1968).
3 G.S. Berenson, P.S. Pargaonkar, S.R. Srinivasan, E.R. Dalferes, J.R. and B. Radhakrishnamurthy, Clin. chim. Acta *56*, 65 (1974).
4 L.A. Carlson and L.E. Böttiger, Lancet *1*, 865 (1972).
5 S.O. Frøberg, Metabolism *20*, 1044 (1971).
6 P.D. Gollnick, Am.J. Physiol. *205*, 453 (1963).
7 C.G. Hames and B.G. Greenberg, Am.J. publ. Hlth *51*, 374 (1961).
8 J.A. Herbert, L. Kerkhoff, L. Bell and A. Lopez-S., J. Nutr. *105*, 718 (1975).
9 A. Lopez-S, A. René, L. Bell and J.A. Herbert, Proc. Soc. exp. Biol. Med. *148*, 640 (1975).
10 P.D. Wood, W. Haskell, H. Klein, S. Lewis, M.P. Stern and J.W. Farquhar, Metabolism *25*, 1249 (1976).
11 J.W. Gofman, H.B. Jones, F.T. Lindgren, T.P. Lyon, H.A. Elliott and B. Strisower, Circulation *2*, 161 (1950).
12 D.P. Barr, E.M. Russ and H.A. Eder, Am.J. Med. *11*, 480 (1951).
13 E. Nikkilä, Scand. J. Clin. Lab. Invest. *5* (suppl. 8), 76 (1953).
14 C. Allard and G. Choquette, Can. J. Biochem. Physiol. *40*, 1515 (1962).
15 G.J. Miller and N.E. Miller, Lancet *1*, 16 (1975).
16 N.E. Miller, O.H. Førde, D.S. Thelle and O.D. Mjøs, Lancet *1*, 965 (1977).
17 W.P. Castelli, J.T. Doyle, T. Gordon, C.G. Hames, M.C. Hjortland, S.B. Hulley, A. Kagan and W.J. Zukel, Circulation *55*, 767 (1977).
18 A. Lopez-S, R. Vial, L. Balart and G. Arroyave, Atherosclerosis *20*, 1 (1974).
19 E.W. Rasmussen and A.T. Høstmark, Circulation Res. *42*, 598 (1978).
20 A.T. Høstmark and R. Askevold, Artery *4*, 129 (1978).
21 J.V. Bradley, in: Distribution-free statistical tests, p. 105. Prentice-Hall, Inc., Englewood Cliffs, N.J. 1968.

Cholecystokinin-like peptides in avian brain and gut

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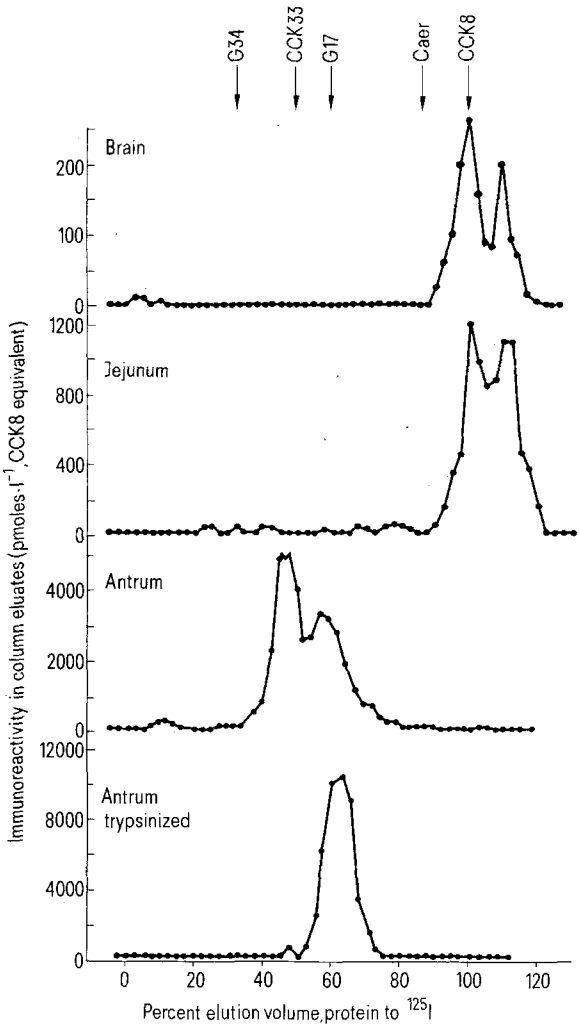
Summary. Extracts of turkey brain and jejunum contain a factor closely resembling the COOH-terminal octapeptide of porcine cholecystokinin (CCK). Turkey antral extracts contain factors distinguishable in immunochemical and gel filtration properties from the mammalian forms of gastrin and CCK.

In mammals, gastrin and cholecystokinin (CCK) occur in both gut endocrine cells and central or peripheral neurones, and so may function not only as gut hormones but also as neurotransmitters or neuromodulators[2-5]. Porcine gastrin and CCK share a common COOH-terminal pentapeptide, and on this evidence are thought to have evolved from a common ancestor[6,7]. However, the phylogenetic origins of the dual distribution in brain and gut remain poorly understood. In the present study the comparative aspects of this problem have been extended to include an immunochemical analysis of gastrin- and CCK-like peptides in the brain and gut of a bird, the turkey *(Meleagris gallopavo).*

Methods. Young turkeys (0.5–4.0 kg) were anaesthetized with urethane, and brain, proximal jejunum and antrum removed and boiled for 3 min in water or acetic acid (0.5 M). The tissue extracts (0.1 g/ml) were homogenized, centrifuged (2000×g, 10 min) and supernatants stored at −20 °C prior to analysis by radioimmunoassay (RIA) and gel filtration on Sephadex G50. The antiserum (L48) used in routine RIA was raised against synthetic COOH-terminal octapeptide of porcine CCK (CCK8) coupled to bovine serum albumin by carbodiimide[8], and was employed with

Concentration of immunoreactivity in extracts of turkey antrum, brain and jejunum measured with 3 antisera and referred to natural human unsulphated G17 or synthetic CCK8 standard

Tissue	Standard	Antiserum L48	1296	L2
Antrum (nmoles/ml)	G17	1.10	0.17	0.10
	CCK8	1.40	1.27	6.8
Brain (pmoles/ml)	G17	8.20	1.37	0.22
	CCk8	10.7	10.30	8.30
Jejunum (pmoles/ml)	G17	2.50	0.32	0.09
	CCK8	3.50	2.40	2.60



Fractionation on Sephadex G50 superfine (1 × 100 cm) of turkey extracts. Extracts of jejunum were prepared from a 5–10-cm segment taken just distally to the opening of the bile and pancreatic ducts; the antrum was identified as a narrow band of mucosa between duodenum and gizzard after opening the duodenum lengthwise. Columns were equilibrated and eluted with 0.02 M sodium barbitone, pH 8.4, containing 0.02% sodium azide, at 4 °C. Elution volume is expressed as percentage from void volume (0%) estimated by absorption at 280 nm, to ^{125}I (100%). Samples were fortified with bovine serum albumin and $Na^{125}I$ before application to the columns to provide markers for the void and salt regions, respectively. Column eluates were assayed with L48 (1:75,000) using CCK8 standard and ^{125}I CCK8 label. In separate runs the columns were calibrated with pure natural human G34 and G17, pure natural porcine CCK33, and synthetic CCK8 and caerulein; elution volumes of the standards are indicated by arrows. Antral extracts (0.1 g/ml, 1.0 ml) were digested with trypsin (Worthington, TPCK treated; 50 µg/ml, 15 min, 23 °C) and inactivated by boiling.

^{125}I labelled CCK8. In this assay, CCK8, human heptadecapeptide gastrin (G17) and intact porcine CCK (CCK33) cross-react almost equally, indicating specificity of L48 for the common COOH-terminal pentapeptide of gastrin and CCK. In other assays use was made of several gastrin antisera (1295, 1296, L2, L6) of known, but varying specificity[9–11].

Results and discussion. With L48 and CCK8 standard, the total immunoreactivity in extracts of turkey antrum was 8.2±2.3 nmoles/g (mean±SEM, n=4); similar concentrations of gastrin occur in mammalian antral mucosa. Fractionation of the antral extracts on Sephadex G50 revealed 2 peaks of activity (figure). One eluted in a similar position to G17, the other slightly earlier. When antral extracts were digested with trypsin the 1st peak disappeared and there was a corresponding increase in the 2nd peak (figure). Negligible amounts of activity eluted in the position of human big gastrin (G34). However, there is evidence that in pig, G17 is produced by tryptic-like cleavage of G34 during gastrin biosynthesis[12], and the relationships between the 2 components in turkey antrum may therefore resemble those of porcine G34 and G17.

Total immunoreactivity (L48, CCK8 standard) in jejunal and brain extracts was 110±pmoles/g (n=3) and 140±20 pmoles/g (n=3), respectively; similar concentrations of CCK-like immunoreactivity occur in mammalian brain and intestine[2,13]. A major component in both extracts emerged from Sephadex G50 in a similar position to CCK8 (figure), but there was also a substantial peak eluting just after this component. Acid extracts of turkey jejunum and brain contained about 10% immunoreactivity compared with boiling water extracts; since porcine CCK33 is extracted in acid but not water[13] it seems that CCK33-like factors are of negligible importance in brain or gut of birds.

Antisera specific for the NH_2-terminus of G17 (1295)[9], or for intact G17 (L6)[11], failed to reveal activity in extracts of turkey brain or gut. In contrast, COOH-terminal specific antisera (1296, L2, L48) revealed activity in all extracts tested, although estimates of activity in the antrum varied 5–10-fold between antisera when referred to either G17 or CCK8 standards (table). If the avian antral factor was identical with G17 or CCK8 all antisera would give the same values when read from the appropriate standard. The results suggest, then, that turkey antral factors have a COOH-terminal region resembling, but not identical to human G17 or CCK8. However, antisera 1296, L2 and L48 gave similar estimates of activity in brain and jejunal extracts using CCK8 standard (table). Thus in turkey, as in mammals[2,5,13], CCK8 or closely related peptides occur in relatively high concentration in both brain and gut.

Gastrin-like biological activity and immunoreactivity has been described in chicken duodenum[14–17]. With the exception of the paper by Larsson et al.[14], these reports make no mention of activity in the antrum which is an inconspicuous region and might have been overlooked. CCK8 has weak gastrin-like biological activity and cross-reacts to some extent with most gastrin antisera, so that the CCK8-like factors found here may account for previously reported gastrin-like activity of avian intestine.

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2 G.J. Dockray, Nature *264*, 568 (1976).

3 K. Uvnas-Wallensten, J.F. Rehfeld, L.-I. Larsson and B. Uvnas, Proc. natl Acad. Sci. USA *74*, 5707 (1977).
4 J.F. Rehfeld, Nature *271*, 771 (1978).
5 G.J. Dockray, R.A. Gregory, J.I. Harris, J.B. Hutchinson and M. Runswick, J. Physiol. *280*, 16P (1978).
6 E.J.W. Barrington and G.J. Dockray, J. Endocr. *69*, 299 (1976).
7 G.J. Dockray, Gastroenterology *72*, 344 (1977).
8 T.L. Goodman, L. Levine and G.D. Fasman, Science *144*, 1344 (1964).
9 G.J. Dockray and J.H. Walsh, Gastroenterology *68*, 222 (1975).
10 G.J. Dockray, L. Best and I.L. Taylor, J. Endocr. *72*, 143 (1977).
11 G.J. Dockray and I.L. Taylor, Gastroenterology *71*, 971 (1976).
12 G.J. Dockray, C. Vaillant and C.R. Hopkins, Nature *273*, 770 (1978).
13 G.J. Dockray, Nature *270*, 359 (1977).
14 L.-I. Larsson, F. Sundler, R. Håkanson, J.F. Rehfeld and F. Stadil, Cell Tiss. Res. *143*, 409 (1974).
15 E.L. Blair, H.S.A. Sherratt and D.D. Wood, Biochem. J. *104*, 54P (1967).
16 H. Ketterer, H.J. Ruoff and K.-Fr. Sewing, Experientia *29*, 1096 (1973).
17 J.M. Polak, A.G.E. Pearse, C. Adams and J.-C. Garaud, Experientia *30*, 564 (1974).

Cytotoxicity of ethyl methanesulfonate in mice spermatogonia

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Summary. Enumeration of different types of spermatogonia, following a single i.p. administration of different doses of ethyl methanesulfonate in mice, showed that survival of A_1–A_4 and in spermatogonia is markedly reduced due to cell killing while the remaining types of spermatogonia were affected marginally. The cell killing effect was dose-dependent, and replenishment of these cells was observed by the end of one cycle of the seminiferous epithelium comprising of 8.5 days.

According to the latest concept of Oakberg[1,2] and Huckins[3], the stem cell spermatogonia duplicate in succession to produce different types of spermatogonia in the seminiferous epithelium of mice testes. Radiation, as well as a number of mutagenic and/or carcinogenic chemicals, have been reported to be cytoxic to these cells[4–7]. In the present investigation, effect of ethyl methanesulfonate, an alkylating agent, mutagenic in the germ cells of mouse[8–10] and rat[11] was evaluated in relation to the specific spermatogonial cell killing.

Material and methods. Adult Swiss mice were given a single i.p. injection of different doses (100, 200, 300 and 400 mg/kg b.wt) of ethyl methanesulfonate (EMS) in normal saline and the control group received 0.5 ml of the vehicle. The mice were killed at intervals of 1, 3, 5 and 8.5 days post treatment. Deparaffinized sections of testes, fixed in Zenker formal, were stained with periodic acid-Schiff reagent and counterstained with hematoxylin. Differentiating spermatogonia [A_1–A_4, intermediate (In) and B] including stem cell (As) spermatogonia and preleptotene spermatocytes (Pl) were enumerated from comparable samples of tubules of seminiferous epithelium in control and treated mice. Results have been expressed as experimental/control ratio[1,2].

Effect of ethyl methanesulfonate on various types of mice spermatogonia

Days after EMS injection	Types of spermatogonia* A_S	A_1	A_2–A_4	In	B	Pl
Control	51	41	162	102	234	395
Dose 100 mg/kg b.wt						
1	39 (0.77)	24 (0.59)	142 (0.88)	93 (0.91)	197 (0.84)	317 (0.80)
3	47 (0.92)	30 (0.73)	118 (0.73)	73 (0.72)	179 (0.77)	370 (0.94)
5	47 (0.92)	21 (0.51)	91 (0.56)	76 (0.75)	178 (0.76)	308 (0.78)
8.5	49 (0.96)	25 (0.61)	122 (0.76)	75 (0.74)	174 (0.74)	389 (0.99)
Dose 200 mg/kg b.wt						
1	38 (0.80)	35 (0.86)	158 (0.98)	101 (0.99)	196 (0.84)	390 (0.99)
3	38 (0.80)	27 (0.66)	133 (0.82)	68 (0.67)	191 (0.82)	380 (0.96)
5	39 (0.76)	30 (0.73)	139 (0.86)	76 (0.75)	213 (0.91)	386 (0.98)
8.5	54 (1.06)	27 (0.66)	93 (0.57)	64 (0.63)	126 (0.54)	384 (0.97)
Dose 300 mg/kg b.wt						
1	35 (0.69)	19 (0.46)	46 (0.28)	38 (0.37)	80 (0.34)	352 (0.89)
3	25 (0.49)	10 (0.24)	21 (0.13)	12 (0.12)	12 (0.05)	28 (0.07)
5	25 (0.49)	19 (0.46)	20 (0.12)	11 (0.11)	30 (0.13)	96 (0.24)
8.5	41 (0.80)	22 (0.53)	95 (0.59)	86 (0.84)	187 (0.80)	384 (0.97)
Dose 400 mg/kg b.wt						
1	27 (0.53)	15 (0.37)	38 (0.24)	37 (0.36)	84 (0.36)	352 (0.89)
3	31 (0.61)	11 (0.27)	17 (0.11)	1 (0.01)	2 (0.01)	4 (0.01)
5	32 (0.63)	21 (0.51)	23 (0.14)	6 (0.06)	15 (0.06)	2 (0.01)
8.5	52 (1.02)	18 (0.44)	107 (0.66)	86 (0.84)	173 (0.74)	352 (0.89)

*Spermatogonia type A_S includes A-pair and A-align, counted from seminiferous tubular stages 1 through 12; A_1 spermatogonia from stage 1 to 9; A_2 to A_4 type spermatogonia from stage 10 through 2; In (intermediate) in stage 3 and 4; B type spermatogonia in stage 5 and 6 and Pl (preleptotene spermatocytes) in stage 7 only. From each mouse various types of spermatogonia were counted from 99 tubular cross sections. Each set of results is the average number of cells counted from at least 2 mice and also expressed as mean experimental/control ratios (values shown within parenthesis).