Table 1. Body weights (grams) of rats injected with cholesterol-26-^{14}C (means ± S.E.). Numbers of rats appear in parentheses. By *t*-test: Sprague-Dawley versus Wistar, $P < .01$; males versus females (Sprague-Dawley), $P < .05$.

Strain	Control	Experimental	Males	Females
Sprague-Dawley	217.5 ± 16.5 (6)	207.5 ± 12.8 (12)	231.1 ± 15.6 (9)	190.6 ± 8.6 (9)
Wistar	150.5 ± 7.2 (10)	149.5 ± 4.7 (10)	156.0 ± 7.0 (10)	144.0 ± 4.1 (10)

Powder Co.) and 2.5 μc of cholesterol-26-^{14}C (Nuclear-Chicago, Chicago, Ill.; specific activity, 24.0 mc/mmole). The optically clear preparation was injected into the tail vein. The rats were immediately placed in a specially designed airtight drum having a diameter of 30 cm and a total volume of 7.2 liters. Air free of CO_2 was admitted to the drum and then aspirated at a rate of 1.2 liter/min and bubbled into 15 ml of a 1 : 2 (by volume) mixture of ethanolamine and ethylene glycol monomethyl ether (*5*).

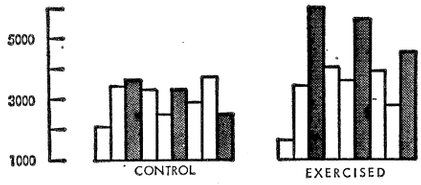

Fig. 1. Typical results showing ^{14}C (disintegrations per minute) in the expired air from two Sprague-Dawley rats. Columns correspond to consecutive 20-minute collection periods starting 15 minutes after intravenous injection of 2.5 μc of cholesterol-26-^{14}C. Stippled columns, rotation of the treadmill; open columns, rest periods.

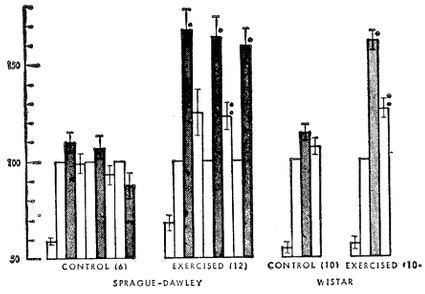

Fig. 2. Average ^{14}C (arbitrary units) in the expired air from rats. Collection of CO_2 was started 15 minutes after intravenous injection of 2.5 μc of cholesterol-26-^{14}C. Columns correspond to consecutive 20-minute collection periods; bars, standard errors of the means. Stippled columns, rotation of the treadmill; open columns, rest periods. The period immediately preceding rotation of the treadmill is taken to equal 100 for the initial as well as for the following two periods. Numbers of animals appear in parentheses. For the Sprague-Dawley rats the number of determinations for each period equals the number of animals; for the Wistar rats there were 16 rotation and 14 postrotation periods for the controls, and 16 activity and 12 postactivity periods for the exercised animals. Differences (*t*-test) between corresponding periods for control and experimental animals: $P < .001$(*), $P < .01$(**).

Fifteen minutes after the injection, expired CO_2 was collected for four to nine 20-minute periods; every third period the rat was exercised by rotation of the treadmill at a rate of 15 to 17 rev/min. Control animals were immobilized and suspended on the axis of the drum; the treadmill was rotated and CO_2 was collected as before. Fifteen milliliters of a scintillation solution (*5*) was added to 3 ml of collecting fluid, and the radioactivity was assayed in a Packard Tri-Carb liquid-scintillator spectrometer (model 3003).

Body weights and sex distribution (Table 1) were similar in control and experimental animals. Since the response to treadmill activity was the same in males and females, the results for both sexes are reported together for each strain. Results of typical experiments are shown in Fig. 1. Figure 2 shows the ^{14}C radioactivity in the expired air from six control and 12 experimental Sprague-Dawley rats. So that all animals could be compared, the disintegrations per minute for the period immediately preceding rotation of the treadmill have been taken to equal 100.

One sees that rotation of the treadmill caused no significant changes in the controls. In the experimental animals, however, treadmill activity led to increased oxidation of cholesterol, which persisted into the period immediately following activity. Oxidation was consistently increased in each animal during the three activity periods. Results with Wistar rats were essentially similar (Fig. 1).

Despite the indications that disturbed metabolism of cholesterol may be implicated in the genesis of atherosclerosis (*6*), we do not know what mechanisms influence the possible inverse relation between occupational activity and occlusive arterial disease (*7*). Our results clearly demonstrate accelerated oxidation of cholesterol, induced by treadmill activity in rats. If our findings prove to be true for other species, one may speculate that physical exercise may retard development of atherosclerosis by increasing the removal of cholesterol by oxidation.

MANUEL R. MALINOW
PHYLLIS MCLAUGHLIN, ANNE PERLEY
Oregon Regional Primate Research Center, Beaverton 97005

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18 April 1968

Secretory Immunoglobulin A: Autoantibody Activity in Gastric Juice

Abstract. *An immunoglobulin A of the secretory variety, present in the gastric juice of a patient with pernicious anemia, was shown to have specificity for intrinsic factor. This is the first demonstration in gastric juice of antibody activity restricted to secretory IgA; further, this is the first example of an exocrine (gastric) immune system producing an autoantibody specifically directed toward a product synthesized by that same exocrine organ.*

Immunoglobulin A (IgA) of exocrine origin (saliva, gastrointestinal fluids) differs from serum IgA in that it contains an additional nonimmunoglobulin component, the so-called secretory piece (*1, 2*). As shown by Tomasi *et al.*, the complex of IgA and secretory piece, referred to as secretory IgA, is unique to exocrine fluids (*1*). Isoagglutinin antibody activity has been demonstrated in secretory IgA of salivary secretions (*1*), but an autoantibody of this variety has hitherto not been reported. This paper describes a secretory IgA autoantibody to intrinsic

factor present in the gastric juice of a patient with pernicious anemia.

Antiserums used in these studies included rabbit antiserums previously shown to be monospecific for human IgG, IgA, and IgM (*3*). The antiserum to secretory (salivary) IgA was that previously used by South *et al.* (*2*). We absorbed this antiserum with human serum obtained from a subject with a selective absence of IgA (normal IgG and IgM); when tested in Ouchterlony immunodiffusion against colostral IgA and myeloma IgA, a pattern of partial identity was observed, the colostral IgA precipitin band spurring over the myeloma IgA band. The antiserum was further absorbed with lyophilized myeloma IgA thereby rendering it nonreactive with serum IgA, but reactive with secretory piece.

Gastric juice was obtained from a healthy adult and from the patient (V.A.) by naso-gastric intubation after histamine stimulation. Pepsin was inactivated by adjusting the *p*H to 10 for 20 minutes; the *p*H was then titrated to neutrality, and the gastric juice stored at −20°C. Intrinsic factor was measured by a charcoal assay (*4*) and expressed as picograms of vitamin B_{12} labeled with Co^{60} ($B_{12}Co^{60}$) bound by 0.1 ml of gastric juice. The patient's gastric juice was initially concentrated to final volume of 2.5 ml by negative-pressure dialysis at 4°C. This concentrated gastric juice was tested in Ouchterlony immunodiffusion with rabbit antiserums monospecific for IgG, IgA, and IgM. It was also tested in immunodiffusion against antiserum to secretory IgA; the adjacent wells were filled with myeloma IgA, colostral IgA, and the human serum with selective absence of IgA. Binding antibody to intrinsic factor was tested for by using the radioimmunodiffusion technique (*5*). Intrinsic factor was complexed to B_{12}-Co^{60} by incubation of 0.5 ml of normal gastric juice (intrinsic factor content 3000 pg per 0.1 ml) with 22,000 pg of $B_{12}Co^{60}$ for 10 minutes at room temperature. Free (noncomplexed) B_{12}-Co^{60} was removed by absorption with albumin-coated charcoal. Rabbit antiserums (0.05 ml), monospecific for either IgG, IgA, IgM or secretory piece, was added to 0.1 ml portions of the complex. Each mixture was diffused in agar against the concentrated gastric juice. Serial twofold dilutions of the concentrated gastric juice were also tested against a mixture of antiserum to secretory piece and the complex. After diffusion for 12 to 16 hours, the slides were washed for 36 hours in saline. The slides were dried on filter paper and placed, agar side down, on Kodak no-screen x-ray film. Films were developed at 3 weeks and examined for radioactive precipitin bands. Controls in each test consisted of concentrated normal gastric juice and of concentrated colostral IgA.

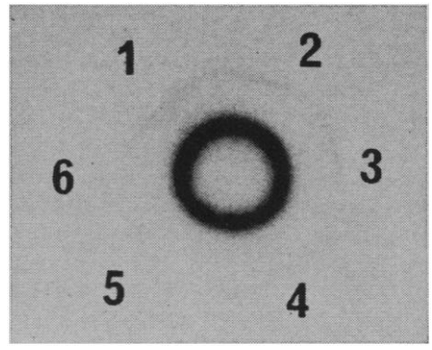

Fig. 1. Radioautograph. Intrinsic factor: $B_{12}Co^{60}$ complex plus antiserum to secretory piece (center well) tested against serial dilutions of patient's concentrated gastric juice: well 1, undiluted; well 2, 1:2; well 3, 1:4; well 4, 1:8; well 5, 1:16. Well 6 contained concentrated normal gastric juice.

Intrinsic factor content of the normal gastric juice was 3600 pg per 0.1 ml; no activity for this factor was detected in the patient's gastric juice. The patient's concentrated gastric juice did not interact with rabbit antiserum to IgG or antiserum to IgM in agar-gel diffusion; a precipitin band, however, was formed when tested against the antiserum to IgA or antiserum to secretory IgA. The IgA was shown to be secretory IgA in that it reacted with antiserum specific for secretory IgA. The precipitin band gave a pattern of complete identity with colostral IgA; the colostral IgA line was partially identical to isolated myeloma IgA. The impure colostral IgA also formed a second faint precipitin band with the antiserum to secretory IgA. After absorption of the antiserum to secretory IgA with myeloma IgA, it gave a single precipitin line when interacted with the patient's concentrated gastric juice, thereby indicating its monospecificity for secretory piece in gastric juice. In contrast to the gastric juice, colostrum gave two precipitin bands with the absorbed antiserum; one represented secretory piece and the second was a component not present in serum or in gastric juice.

Binding antibody to intrinsic factor was detected in the concentrated gastric juice when rabbit antiserum either to IgA or to secretory piece was used as coprecipitating agents (Fig. 1). No antibody activity was detected with antiserums to IgG or IgM. No radioactivity was detected in the single precipitin line formed by the interaction of concentrated normal gastric juice with antiserum to secretory piece; the two precipitin arcs formed by the impure colostral IgA were also nonradioactive.

The results of our experiment document a secretory IgA autoantibody to intrinsic factor. Other investigators have demonstrated autoantibodies to intrinsic factor in the gastric juices of certain patients with pernicious anemia; these, however, all have been IgG immunoglobulins (*6*). An IgA autoantibody to intrinsic factor in saliva of one subject has been described, but it was not studied to ascertain whether it was of the secretory variety; this patient's gastric juice contained an IgG antibody to intrinsic factor (*7*). Our data indicate that an autoantibody of the secretory IgA variety was produced by the gastric immune system. Furthermore, to our knowledge, this is the first demonstration of an exocrine immune system producing an antibody specifically directed toward a product synthesized by that same exocrine organ.

LEONARD S. GOLDBERG
JOSEPH SHUSTER, MARGARET STUCKEY
H. HUGH FUDENBERG
Section of Hematology and Immunology, Department of Medicine, University of California, San Francisco Medical Center, San Francisco

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