these membranes to divalent cations is quite striking: whereas they do not coagulate grossly when concentrated isopycnically in gradients of NaBr (salt concentration $> 1M$), they form large clumps under conditions which are identical except for the presence of $0.001M$ $MgSO_4$ (*11*). The contrasting stability of the plasma-membrane fragments demonstrates an important difference between the electrical properties of membranes arising from the external surface of Ehrlich ascites carcinoma cells and those arising from the cell interior.

The plasma-membrane material in the top zone can be further purified by centrifugation in Ficoll density gradients. To do this, the material of the top zone was adjusted to appropriate density and mixed directly into Ficoll gradients containing $0.001M$ Tris, *p*H 8.6. The gradients were centrifuged in an SW-39 rotor at 35,000 rev/min for 16 hours at 4°C (Figs. 1*b* and 2). There are two principal components: a small one with a density of 1.024 at 4°C and a large one with a density of 1.050. The region between these two bands, not homogenous, consists of a series of closely spaced layers which extend into the peak at 1.050 density. The low-density component, which scatters light strongly, is rich in DPNH-diaphorase, but lacks surface antigen and Na^+- and K^+-activated adenosine triphosphatase.

The distribution of surface antigen and Na^+- and K^+-activated adenosine triphosphatase shows in three experiments that the plasma-membrane vesicles are located in the larger band with modal density at 4°C of 1.050 ± 0.001, a density considerably less than that found from distributions obtained with whole microsomal membranes (*4*). This suggests that the previous values were spuriously high because of nonspecific interactions between vesicles of different origins. The DPNH-diaphorase in the plasma-membrane zone may be due to (i) continued contamination with membranes arising only from the cell interior; (ii) presence of vesicles composed of "mixed" membranes, that is, arising from sites where the cell surface and endoplasmic reticulum were continuous at the moment of cell rupture; or (iii) engulfing of fragments of endoplasmic reticulum within large plasma-membrane vesicles at the time of cell rupture.

The striking layer formation throughout the plasma-membrane zone must be taken to indicate discontinuity in the volume or matrix density of plasma membrane-vesicles or both. Volume discontinuity appears more likely, since vesicle volume is a major determinant of density in Ficoll gradients (*4*) and since layers are not observed in gradients of sodium bromide.

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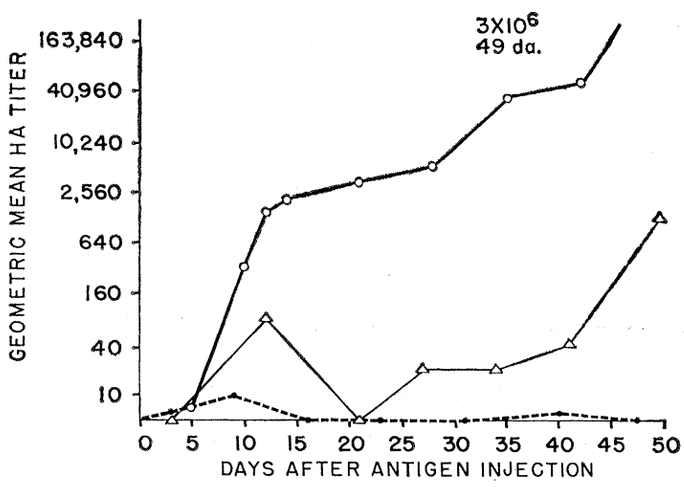
Passive Transfer of the Action of Freund's Adjuvant by Serum of Rabbits Injected with the Adjuvant

Abstract. *Serum collected at intervals from rabbits treated with Freund's complete adjuvant was injected together with fluid diphtheria toxoid into recipient rabbits. Control rabbits received toxoid alone or toxoid mixed with serum of untreated donors. There was no antibody response in the controls or in recipients of serum obtained from donors 3 days after adjuvant treatment. Recipients of serum obtained from donors 1 to 9 weeks after adjuvant treatment responded with antibody formation. The magnitude of the response of recipients was correlated with the increase in γ-globulin concentration in the serums of the corresponding donor rabbits. Passive transfer of adjuvant action indicates that a serum factor, possibly natural antibody, is partially responsible for the immunity-enhancing activity of Freund's adjuvant.*

The theory of antibody formation proposed by Jerne (*1*) maintains that natural antibody, formed in the absence of antigenic stimulation, is required for antibody response. The natural antibody functions as the immunologic recognition system. This view is supported by the work of Segre and co-workers (*2*) on the enhancement by specific antibody of the antibody response in pigs deprived of colostrum. Terres and Wolins (*3*) also reported that specific antibody enhanced the antibody response in mice. One of the consequences of the theory would be that if the concentration of natural antibody is increased, within limits, the magnitude of the antibody response should increase, since more of the injected antigen would combine with antibody and form complexes capable of stimulating the antibody-producing cells. The secondary response may be explained in this manner (*1, 4*).

Since the antibody response to the administration of an antigen mixed with an adjuvant assumes some of the characteristics of a secondary response, the hypothesis may be formulated that adjuvants increase the concentration of natural antibody. Such action may be expected if the adjuvant caused either or both of the following: (i) leakage of natural antibody from lymphoid cells into the circulation, through destruction of the cells or alteration of their permeability; (ii) rapid proliferation of lymphoid cells which produce natural antibody.

Certain findings appear to agree with this hypothesis. Humphrey (*5*) reported that in rabbits injected with Freund's complete adjuvant alone there was an increase in the concentration of circulating γ-globulin, in that about 10 mg of γ-globulin per milliliter of serum could not be accounted for by antibodies directed against *Mycobacterium* and a number of other antigens. Paraf and Moraillon (*6*) injected rabbits repeatedly with Freund's complete adjuvant. When such rabbits were given human serum albumin within 10 days after the last injection of adjuvant, their antibody response was greater than that of rabbits not receiving prior treatment



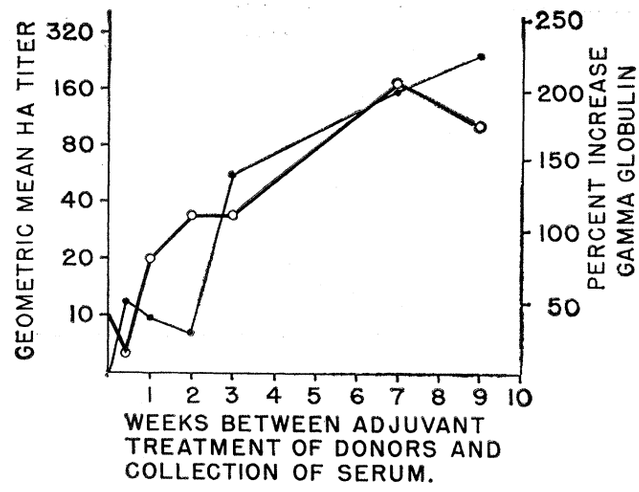


Fig. 1 (left). Antibody response of rabbits given 1 ml of diphtheria toxoid mixed with: Freund's adjuvant (○———○); serum of rabbits treated with Freund's adjuvant alone 7 weeks previously (△———△); or normal rabbit serum (●·····●). Fig. 2 (right). Relation of peak antibody titers of recipients (○———○) to percentage increase γ-globulin in donor serum (●———●).

with adjuvant and was characteristic of a secondary response. Svet-Moldavsky and Raffkina (*7*) reported that Freund's complete adjuvant caused an increase in the number of plasma cells in lymph nodes of rats.

If adjuvants do indeed act according to the proposed hypothesis, then it should be possible to transfer passively the adjuvant action by treating a normal animal with an antigen mixed with serum obtained from animals treated with adjuvant only. Passive transfer of Freund's adjuvant action was obtained in the experiments reported here.

Young donor rabbits (mixed breed, 1.6 to 2.2 kg) were injected subcutaneously at multiple sites, with 2 ml of a mixture of equal parts of Freund's complete adjuvant (*8*) and 0.85 percent NaCl solution. Three days, 1 week, and 2, 3, 7, and 9 weeks after inoculation, groups of four donors were exsanguinated, and their serums were collected. Separate groups of two rabbits each were used as recipients of the serum collected at each interval (Table 1).

Since the amount of natural antibody present in the serums of the adjuvant-treated rabbits would be relatively low and since only about one-half of the total blood volume can be recovered by exsanguination, the serums of two donors were used for each recipient. This procedure would approximate the transfer of all of the serum of a single donor to a recipient.

One-half of the serum given to each recipient was mixed with the antigen [1 ml of fluid diphtheria toxoid, 50 Lf (flocculating) units per milliliter] (*9*) and incubated at 37°C in a water bath for 30 minutes. This mixture was then injected intraperitoneally into the recipient rabbit. The other half of the serum was administered intraperitoneally to the recipient in four equal doses over the next 4 days. Two control rabbits received only the antigen, and two others received the antigen mixed with normal rabbit serum (Table 1). Antibody titers of recipient and control rabbits were determined by the tanned-cell hemagglutination test (*10*) over a period of 50 days after injection.

The control rabbits, receiving antigen alone or antigen mixed with normal rabbit serum (groups 1 and 2, Table 1), showed no significant antibody response. Antibodies to diphtheria toxoid, with titers significantly ($P < .01$) greater than those of the control rabbits, were found in all recipients of serum of adjuvant-treated donors, except those receiving serum obtained from donors 3 days after adjuvant treatment (group 3, Table 1). The antibody response was greatest in the recipients of serum obtained 7 weeks after adjuvant treatment of the donors (group 7, Table 1 and Fig. 1).

Table 1. Quantities of donor serum injected in recipient rabbits to effect passive transfer of Freund's adjuvant action and times elapsed between adjuvant treatment and exsanguination of donor rabbits. Four donor and two recipient rabbits were used in each group, except group 1, in which the two recipient rabbits received toxoid but no serum.

Group No.	Time between adjuvant treatment and exsanguination of donors (days)	Donor serum to each recipient (ml)
1		0
2	0	60
3	3	50
4	7	64
5	14	56
6	21	76
7	49	68
8	63	86

From these experiments it was concluded that the action of Freund's adjuvant was passively transferred to the recipients with the serum of the donors. When peak antibody titers of the recipients (*11*) were plotted against the interval between adjuvant treatment and collection of the donors' serum (Fig. 2), it became apparent that the magnitude of the antibody response increased gradually and reached a maximum in the recipients of the 7-week serum. The curve so obtained presumably reflects the concentration of the factor responsible for passive transfer. Since we assumed that "adjuvant activity" of donor serums was a function of the concentration of natural antibody globulin, a correlation was sought between the magnitude of the antibody response of the recipients and the concentration of γ-globulin in the donor serum. The serums of the donor rabbits were analyzed by paper electrophoresis prior to adjuvant treatment and at the time of exsanguination. The total protein content of each serum sample was measured by the biuret method, and the concentration of γ-globulin was calculated. The variation in serum γ-globulin concentration within each group of donors, expressed as percentage increase in the final sample as compared to that obtained prior to administration of adjuvant, was plotted against the interval

of time elapsed between adjuvant treatment and collection of the donors' serums (Fig. 2). The similarity between the curve so obtained and that formed by the peak antibody titers of the various groups of recipients is apparent.

The finding that adjuvant action can be passively transferred, in part, by serum does not contradict the widely held view that Freund's adjuvant acts by slowly releasing antigen over a prolonged time. Such a mechanism of action and the one proposed here are not mutually exclusive. The most important implication of the passive transfer experiments is that they provide additional support for an extracellular control of antibody production exerted by antibody itself. This role of antibody was originally proposed by Jerne (*1*) and further elaborated by Karush, and by Eisen and Karush (*4*). Eisen and Karush postulated that the equimolar antigen-antibody complex constitutes the only effective stimulus for antibody formation. Complexes formed in antigen excess or in antibody excess would not stimulate the antibody-producing cells. The former would cause immunologic tolerance, whereas the latter would "shut off" antibody production when antibody concentration has become sufficiently great (*12*). The concentration of equimolar antigen-antibody complexes would control the magnitude of the antibody response. Such concentration, in turn, depends on the concentration of antibody present in circulation as either natural or immune antibodies. Work from this laboratory has demonstrated enhancement of antibody production in colostrum-deprived baby pigs by low concentrations of homologous and heterologous immune antibodies (*2*) and by large quantities of normal colostrum (*2*) and normal γ-globulin (*13*) which presumably contained natural antibodies. The results reported here suggest that the enhancement of antibody response caused by Freund's adjuvant may also be explained on the basis of an increase in concentration of natural antibodies.

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Pancreatic Secretion Induced by Stimulation of the Pyloric Gland Area of the Stomach

Abstract. *Pure gastrin I of Gregory and Tracy stimulated secretion not only of gastric acid but also of pancreatic fluid and protein material. Similarly, endogenous release of gastrin from a transplanted pouch of the pyloric gland area of the stomach, initiated by irrigation with solutions of acetylcholine, stimulated gastric acid secretion and pancreatic flow and protein output in dogs with fistulas of both organs. The effect on the pancreas, like that on the stomach, was inhibited by acidification of the acetylcholine solution.*

The external secretion of the pancreas in man and other mammals is thought to be controlled by (i) the release of two hormones, secretin and pancreozymin, from the mucosa of the small intestine, and (ii) the nerve supply to the gland (*1*). Although it has been known for several years that extracts of the mucosa of the pyloric gland area of the stomach also possess some stimulatory properties for pancreatic secretion (*2*), the effect has simply been ascribed to the presence of some secretin-like material in this region.

Recently, Gregory and Tracy (*3*) described the isolation of two pure polypeptides from the mucosa of the pyloric gland area, which, by reason of their effects in stimulating gastric secretion, are thought to represent gastrin, the hormone of the gastric phase of gastric secretion. These polypeptides stimulate pancreatic secretion (*3*), suggesting that in the intact animal liberation of gastrin by the presence of food in the pyloric gland area provides a third humoral stimulus for external pancreatic secretion.

If this hypothesis, based upon the pharmacological effects of mucosal extracts on pancreatic secretion, is to be accepted, it is necessary to demonstrate that stimulation of the pyloric gland area in the living animal will release a humoral stimulant of pancreatic secretion. Blair *et al.* (*4*) found that the presence of various stimulants in the pyloric gland area of the anesthetized cat caused an increase in enzyme secretion by the pancreas after the vagus nerves and the splanchnic nerves were cut. These results suggest that stimulation of the pyloric gland area results in the release of a humoral stimulus for pancreatic secretion.

The results of our experiments with conscious dogs offer proof of the existence of a humoral stimulant of pancreatic secretion originating in the pyloric gland area. In these animals a permanent pancreatic fistula was created surgically according to a method we have described (*5*). A pouch was made of the pyloric gland area and drained to the exterior by a mucocutaneous fistula on the abdominal wall. Several weeks later the mesenteric attachments of this pouch were divided, and it became dependent on new vessels growing from the abdominal wall for its blood supply. Thus the pouch could be considered totally denervated. The dogs were also equipped with a gastric fistula draining the remainder of the stomach. With the gastric fistula open, an intravenous infusion of histamine dihydrochloride (4.0 mg/hr) caused a marked increase in gastric secretion but no sustained increase in pancreatic secretion. This demonstrated to our satisfaction that, with the gastric fistula draining freely, any increase in pancreatic secretion in those instances in which gastric secretion was stimulated could not be attributed to the escape of gastric juice into the intestine with subsequent release of secretin.

The effect in these animals of a single subcutaneous injection of pure gastrin I (*6*), 2 μg/kg body weight, is