Elicitation of Arthus Reactions in Guinea Pigs by Homologous γ_1 and γ_2 Immunoglobulins (34652)

J. L. MAILLARD AND G. A. VOISIN
(Introduced by C. A. Krakower)

Département d'Immuno-Pathologie Expérimentale[1] *du Centre d'Immuno-Pathologie de l'Association Claude-Bernard et de l'I.N.S.E.R.M., Hôpital Saint-Antoine, Paris 12ème, France*

The Arthus phenomenon is a remarkable model to study the mechanism of serum antibody mediated immunopathological lesions. The two main pathological features of the phenomenon are edema [and also increased vascular permeability (1–3)], on the one hand, and hemorrhage and necrosis on the other hand. The studies by Ovary and coworkers (4, 5) suggested that, in guinea pigs, the former were due to 7S γ_1 antibodies (endowed with anaphylactic properties), while the latter were due to 7S γ_2 antibodies (endowed with complement fixing properties). On the basis of a previous study showing the role of anaphylaxis (acting through increased vascular permeability) in triggering the Arthus reaction induced with a limited amount of immune serum (2), a hypothesis was elaborated according to which 7S γ_1 antibodies are of prime importance

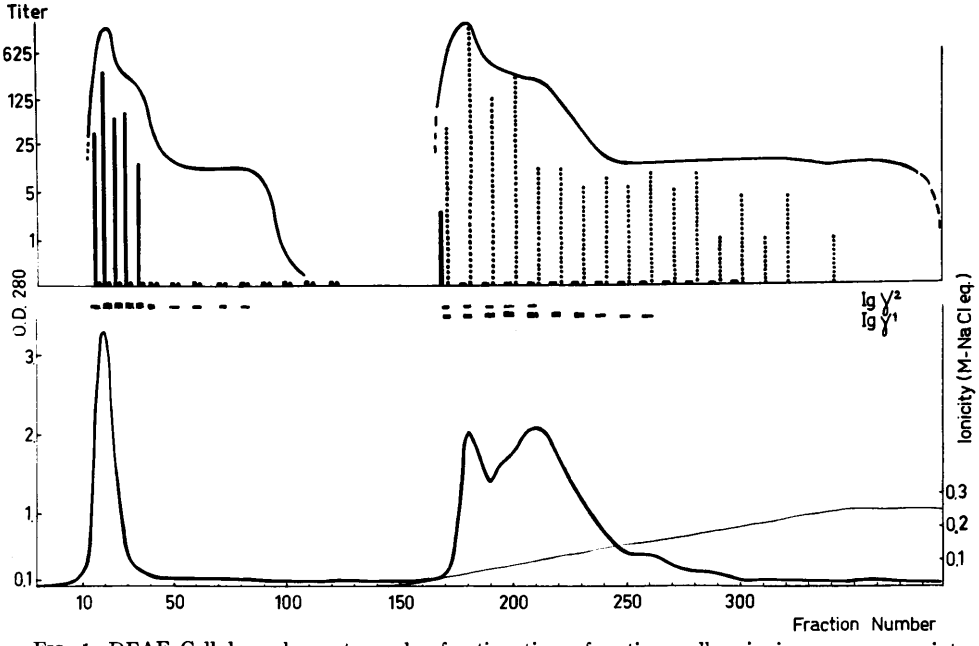

FIG. 1. DEAE-Cellulose chromatography fractionation of anti-egg-albumin immune serum into 7S γ_1 and 7S γ_2 antibodies: Phosphate buffer (0.005 *M*, pH 7.5); gradient by added 0.4 *M* NaCl; (bottom) OD 280 and ionicity gradient; (top) antibodies in fractions with titers in hemagglutination (—); C′ fixation (solid columns); and PCA (dotted columns); (center) scanning in immunodiffusion with anti-immunoglobulin γ_1 and anti-immunoglobulin γ_2 antisera.

[1] Equipe de Recherches Associée au C.N.R.S. (E.R.A. nº 149).

both in triggering and in building up the phenomenon. Experiments were undertaken using better separated immunoglobulins and more rigorous methods than previously done. It was finally shown that, while γ_1 and γ_2 antibodies are necessary to make up a complete, classical Arthus reaction, γ_2 does not elicit any form of lesion by itself; whereas, γ_1 alone can induce a particular form of Arthus reaction which deserves further investigation.

Materials and Methods. An accurate approach to this problem requires firstly to use fractions of sera (purified antibodies are not necessary) containing the two immunoglobulins uncontaminated by each other, as verified by the most sensitive *in vitro* tests, secondly to perform direct passive Arthus reactions to obviate the local inflammation unavoidable after intradermally injected immunoglobulins (5).

Unheated pooled guinea pig sera were fractionated on DEAE-cellulose columns similarly to the method used by Spalter and Ovary (6), omitting prior ammonium sulfate precipitation. A typical case is shown in Fig. 1. The fractions were selected for their purity after a preliminary test by passive cutaneous anaphylaxis (PCA), passive hemagglutination, and passive lysis or C′ fixation; then distributed into several pools, strongly reconcentrated, and tested again. After a final selection and reconcentration of the pools, the γ_1 and γ_2 preparations had fully retained their capacities for passive hemagglutination, hemolysis, anaphylaxis, and precipitation (Table I). They did not contain detectable IgM when studied in a sucrose gradient ultracentrifugation.

Besides exceptions indicated later, the schedule of injections giving rise to maximal reactions was the combination of intravenous γ_1 antibody followed 18 to 24 hr later by intravenous γ_2 antibody given about at the time of the intradermal antigen. The weight of precipitating antibodies injected was known (see Table I). However, on account of the usually high proportion of nonprecipitating antibodies in guinea pigs, the amounts of γ_1 and γ_2 antibodies injected were compara-

TABLE I. Characteristics of γ_1 and γ_2 Antibody Preparations from an Antiegg-Albumin Guinea Pig Serum.[a]

	Vol (ml)	Precipitating activity (mg of antibody protein/ml)	Hemagglutination titer [and yield from initial total activity (%)]		PCA titer [and yield from initial total activity (%)]		Hemolytic titer [and yield from initial total activity (%)]		Reciprocal contamination (%)
Starting material (whole immune serum)	130	3.1	16,000		6500		750		
γ_1 Fraction	43	6.8	12,000	[25]	6500	[33]	0	[0]	<0.1
γ_2 Fraction	24	2.7	8000	[10]	5	[0.02]	1500	[40]	<0.1

[a] These preparations were used for direct passive Arthus reactions.

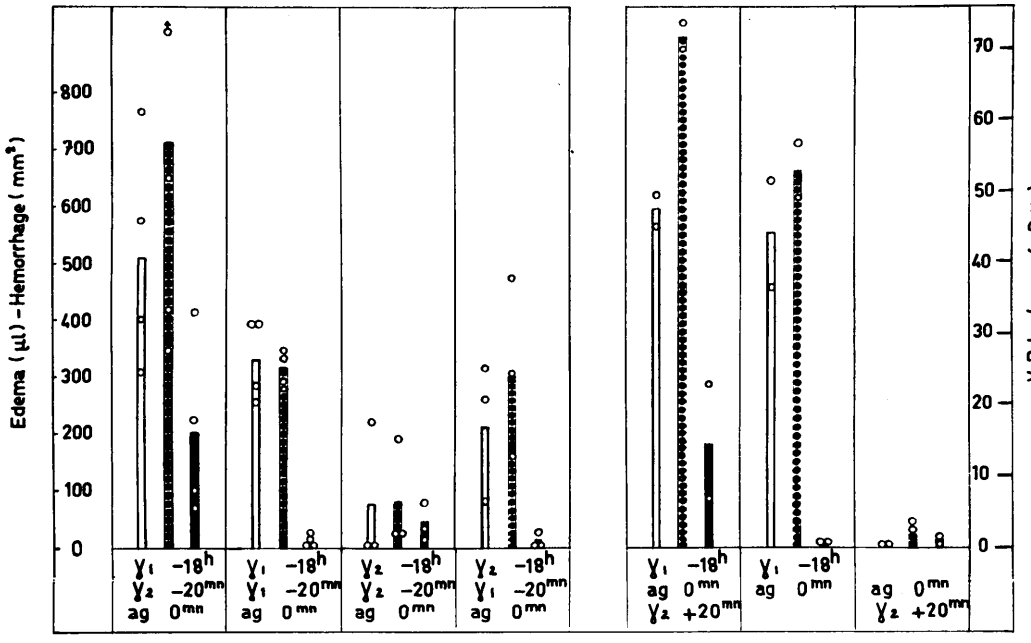

FIG. 2. Role of immunoglobulin γ_1 and immunoglobulin γ_2 in elicitation of Arthus phenomenon: edema (μl) (open columns); vascular permeability increase [surface of the blue spots (mm^2)] (dotted columns); Hemorrhage [surface (mm^2)] (solid columns); (O) individual data; (bottom) order of injections (immunoglobulins are injected iv and antigen id; for more details, see text).

tively measured with the use of hemagglutinating units (hU). For example a dose of 0.5 ml of a preparation giving a titer of 12,000 in passive hemagglutination was expressed as a dose of 6000 hemagglutinating units. Three quantitative characteristics of the reactions were recorded: volume of edema (μl) evaluated by calculations from diameter and thickness of the reactions; surface of hemorrhage (mm^2), and intensity of vascular permeability increase (VPI). For the latter, unless otherwise stated, in order not to test the extravasation of the immediate type but only that of the Arthus reaction, Evans blue was injected 20 min after antigen. The blue spots were estimated for the weight of dye by matching them with spots of known values.

Results. Participation of γ_1 and γ_2 immunoglobulins in the direct passive Arthus reaction. In a typical experiment with an egg albumin–antiegg-albumin system, a combination of 6000 hU (3.4 mg of precipitating antibody protein) of γ_1 and 8000 hU (2.7 mg) of γ_2, injected, respectively, 18 hr and 20 min before 100 μg of antigen, proved capable to give rise to a typical hemorrhagic Arthus reaction. Figure 2 (left side) refers to a comparison of the 3 main characteristics of a 4-hr-old reaction, when in following the preceding schedule for the two antibody injections, a γ_1 injection was replaced by a γ_2 injection and vice versa, or when γ_1 and γ_2 injections were permutated. The amount of antibody on the hemagglutinating unit basis was kept constant in each injection.

It is clearly shown that immunoglobulin γ_1 and immunoglobulin γ_2 are not interchangeable; to give rise to a complete hemorrhagic Arthus reaction requires a special chronology of the 2 injections; immunoglobulin γ_1 alone brings about a strong exudative albeit nonhemorrhagic lesion; immunoglobulin γ_2 by itself is almost ineffective.

In a second series of experiments (Fig. 2, right side) designed to reduce the local consequences of the trauma provoked by the intradermal injection upon circulating γ_2 (*i.e.*, inflammation with increased vascular permeability), the animals received either

one injection of γ_1 18 hr before antigen, or one injection of γ_2 20 min after (and not before as above) the antigen or both γ_1 and γ_2 (γ_1 18 hr before and γ_2 20 min after antigen); the doses were the same as above. The results become more explicit : immunoglobulin γ_1 is able to act alone, immunoglobulin γ_2 is not. The former produces a prominent exudative reaction, upon which the latter confers the hemorrhagic component, thus insuring the completion of the classical Arthus reaction.

In an other study dealing with antihuman serum albumin immunoglobulins, similar although less striking results were obtained. In brief, an appreciable hemorrhage (more than 20 mm^2) occurred in 5 out of 5 guinea pigs receiving a succession of γ_1 and γ_2, 1 out of 4 receiving γ_2 twice, 0 out of 5 receiving γ_1 twice.

Thus no visible local lesion was created by γ_2 antibody alone even in amount equal or superior to the optimal dose defined in a classical study (10). This raised the question of some biological alteration due to *in vitro* manipulations. Two experiments were designed firstly to look for a disparity in the behavior of isolated γ_2 in guinea pig organism as compared to γ_1, secondly to produce Arthus reactions when only unaltered immunoglobulins were in circulation.

In a series of 3 pairs of animals receiving 3 types of antibovine gammaglobulin (BGG) antibodies, *i.e.*, whole immune serum (6400 hU), isolated γ_1 (6400 hU) and isolated γ_2 antibody (12,800 hU), blood samples were serially drawn from 5 min to 48 hr after antibody injection. A comparison of hemagglutinins and hemolysins showed that isolated γ_1 and γ_2 or whole serum antibodies were cleared from blood at a high rate during the early phase (5 min to 8 hr) and at a low rate during the late phase (8 to 48 hr). Taking as reference the hemagglutinating titers in blood at 5 min, the rate of disappearance in the early phase was 60, 60, and 80% for whole serum, γ_1 and γ_2 antibodies, respectively. With reference to the titer in blood at 8 hr, a similar rate of disappearance close to 20% was found for these 3 materials in the late phase.

Relying on the fact that the part of anti-

TABLE II. Characteristics of Arthus Reactions When Antigen Was Injected 48 hr After γ_1 or γ_2 Antibodies or a Combination of Both.[a]

Antibodies injected	Amount (mg) of precipitating antibody in circulation		Antibody titers in sera at 48 hr		Arthus reactions		
	At time of injection	At 48 hr (assumed[b])	Passive hemagglutination	Passive hemolysis	Edema (μl)	VPI (μg of dye)	Hemorrhage (mm^2)
γ_1	1.3[c]	0.52	128, 128[d]	0, 0	354, 426	23, 60	0, 0
γ_2	2.6	0.52	128, 256	128, 128	35, 105	2, 13	7, 3
$\gamma_1 + \gamma_2$	1.08 (γ_1) + 0.52 (γ_2)	0.53 ($\gamma_1 + \gamma_2$)	128, 128	12, 12	461, 346	47, 44	201, 78

[a] The time is chosen beyond the phase of high rate elimination of the isolated antibodies.
[b] Assuming the same rate of elimination for precipitins and hemagglutinins of the same Ig class.
[c] Antibody protein (mg). The immune system is BGG–anti-BGG.
[d] Individual values for the 2 guinea pigs of a group.

bodies remaining at 48 hr had been biologically cleared of the most altered immunoglobulins, we reinvestigated the elicitation of Arthus reactions giving the antigen, BGG, at that time. Moreover, for a better quantitation of the antibodies truly active *in vivo*, the amount of precipitating antibody in form of γ_1, γ_2 or a mixture of both, each modality being tested in a pair of guinea pigs, was adjusted to give every animal an identical concentration of precipitins in blood at 48 hr (assuming a similar elimination rate for precipitins and hemagglutinins of same Ig class). Direct passive reactions were performed as above with blue injected 20 min and sacrifice made 4 hr after antigen. The doses of antibodies are given in detail in Table II. From the results shown in Table II, the intensity and the aspect of the lesion appear to be determined by the type and the association of immunoglobulins in the same manner as previously.

That the two immunoglobulins had to react specifically to play their role in the reaction was demonstrated in additional experiments, by the inefficiency of γ_1 or γ_2 antibody alone to achieve a complete reaction when they had inadequate specificity for the antigen used.

Some drawbacks in our effort to extend the above described findings deserve to be mentioned: an attempt to produce such a complete reaction with two unrelated immune systems comprising antihuman serum albumin γ_1, antiegg albumin γ_2 and a mixture of the corresponding antigens remained unsuccessful. Yet when γ_2-antigen complexes were intravenously injected before initiation of the unrelated γ_1 reaction (in a manner similar to Bier's (7)), the lesion was hemorrhagic.

Characteristics of the exudative Arthus reaction engendered by immunoglobulin γ_1. Being in need of large amounts of isolated γ_1 to undertake the next experiment, we turned ourselves to guinea pig sera directed against autoantigen S (an orchitogenic glycoprotein extracted from guinea pig spermatozoa) which gives rise to mainly γ_1 and almost no γ_2 antibodies both without precipitating capacity (8). After chromatography, the γ_1 antibody recovery amounted to 87% of the hemagglutinins in the original serum with no detectable trace of γ_2 after a quantitative complement fixation test.

The chronologic development of direct passive Arthus reactions created with γ_1 anti-S antibody was followed over a period of 2 days. In every case the antibody (3500 hemagglutinating units) was injected intravenously 24 hr before antigen (a dose equivalent to 20 million spermatozoa). In seven series of 3 to 4 animals each, the injection of Evans blue and the sacrifice were timed respectively as follows (antigen injection on zero time) (i) minus 5 and 20 mn; (ii) 20 mm and 1 hr; (iii) 1 and 2 hr; (iv) 2 and 4 hr; (v) 4 and 8 hr; (vi) 8 and 24 hr; (vii) 24 and 48 hr.

Besides the constant absence of hemorrhage and necrosis as above, the outstanding characteristics were (Fig. 3): a massive edema culminating at 2–4 hr, then subsiding slowly in not less than 24 hr; a prominent vascular permeability increase, the bulk of which occurred in the Arthus period (20 min to 2 hr) and not in the immediate period.

Several features were found unmodified: polymorphonuclear leukocyte and platelet counts in blood, C′ titers, recalcified plasma-coagulation times.

Microscopically, the main events observed in reactions aged 20 min to 48 hr were: an early and intense emigration of polymorphonuclear cells far from the venules followed by their degranulation and death; an extravasation of mononuclear cells contributing to the gradually increasing proportion of emigrated cells; a reduced damage of vascular walls compared to a complete hemorrhagic Arthus.

The main characteristics listed above were constantly observed in various studies with different γ_1 immunoglobulin preparations, as well as with different antigens (human serum albumin, egg albumin, DNP protein, bovine gamma globulin), and different dosages and schedules of injections.

Discussion. The unexpectedly intense inflammation exhibited at the site where γ_1 immunoglobulin and the corresponding antigen react is in line with earlier findings made in this laboratory. For example, when guinea pigs are immunized against human serum albumin and tested with bovine serum al-

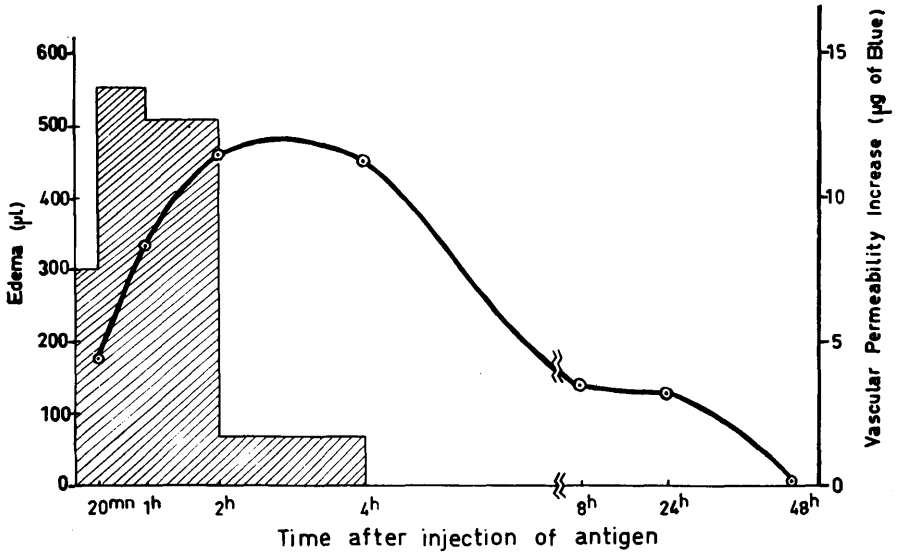

FIG. 3. Development of the exudative arthus reaction (white Arthus) with the immunoglobulin γ_1 anti-S-preparation: edema (—); vascular permeability increase (shaded surface) expressed as the average extravasation of dye between two times; γ_1 antibody was injected 24 hr before antigen.

bumin, or against native transferrin and tested with heated transferrin (9), they display an early lesion which, besides absence of hemorrhage, is similar to the classical Arthus reaction. This particular type of lesion can be reasonably considered as being the consequence of an aggression by the non-C′-fixing immune complexes.

All those findings together with the ones concerning the effect of γ_1 immunoglobulins reported above lead us to the differentiation of a type of immune inflammation brought about by the use of homologous non-C′-fixing antibody, at variance with the classical Arthus reaction. This kind of non-hemorrhagic, non-necrotic, purely exudative reaction was tentatively named exudative Arthus reaction or "white Arthus." That similar features are found in the moderate, hardly hemorrhagic Arthus reaction produced in guinea pigs by the poorly C′-fixing (also non-anaphylactic) horse antibody (10) deserves to be recalled here. The capability of γ_1 antibody–antigen complexes to create a proper variety of inflammation without activating the C′ sequence (*i.e.*, without the help of anaphylatoxin or C′ chemotactic factors) is again confirmed by the absence of inhibition of the exudative Arthus reaction in animals depleted of their C′ by the nonaggressive highly efficient cobra venom C′ inactivator [prepared according to the Nelson procedure (11)]. This inflammation is thought to be distinct from a mere anaphylactic (in the sense of histaminergic) reaction considering its development over several hours in the post-immediate period, its intense microscopic changes and its resistance to antihistaminics. Therefore, one or several agents independent of C′ activation are postulated as mediators of the exudative type of Arthus reaction.

The inability of γ_2 antibody alone to elicit a reaction does not entail a molecular damage during its *in vitro* handling, since biological properties of these immunoglobulins, including precipitation, persist after the processing; the rate of elimination from blood is not more pronounced for γ_2 than for γ_1 antibody, especially in the late phase; finally, a high blood concentration of "biologically filtered" γ_2 antibody allows almost no reaction. The answer to where and when the specific combination of γ_2 antibody and antigen takes place will require fluorescein or isotope-labeled antibody studies and cannot be given at present.

Contradictorily, Bloch *et al.* (5) reported a

full capacity of γ_2 antibody to build up a hemorrhagic reverse Arthus reaction, but a nonspecific triggering can be imputed to the inflammatory effect of intradermally injected immunoglobulins, playing the role of the exudative lesion in our experiments. Closer to our results is a recent experiment by Bier *et al.* (7) showing that γ_1 but not γ_2 antibody could trap circulating complexes in vessel walls.

Another unsolved problem is how the action of γ_2 antibody, unable in itself to build up any lesion is made possible by the prior effect of γ_1 antibody. Do γ_1 immune complexes act by increasing vascular permeability (thus allowing γ_2 antibody to gain access to tissues), or do they localize γ_2 immune complexes in vessels (12)? Alternatively, does a two-step phenomenon reminding us of a Shwartzman reaction (13) take place? Further work is necessary to substantiate one of these possibilities.

Summary. Direct passive Arthus reactions were performed in guinea pigs with homologous γ_1 and γ_2 antibody preparations fully reactive in precipitation, passive hemagglutination, and less than 0.1% reciprocally contaminated (as verified with passive cutaneous anaphylaxis and passive hemolysis). Under these conditions, a definite role for the elicitation of the reaction could be ascribed to each of these two immunoglobulins: γ_1 antibodies alone build up a strongly edematous reaction with vascular permeability increase but no hemorrhage; the hemorrhage is conferred to the preceding reaction by γ_2 antibodies; γ_2 antibodies alone are practically incapable of producing a visible reaction. The lack of reaction with γ_2 antibodies alone was not subsequent to a drastic elimination of this type of immunoglobulin since a similar combination of results was obtained when large quantities of γ_2 antibodies were given 20 min after, 20 min or 48 hr before antigen. In the latter case, their persistence in blood with full biological capacities was verified.

We wish to thank Miss C. Farreau for her valuable assistance.

1. Voisin, G. A., and Glenchur, H., Ann. Inst. Pasteur, Paris **107**, 293 (1964).

2. Voisin, G. A., and Maillard, J., Ann. Inst. Pasteur, Paris, **114**, 173 (1968).

3. Voisin, G. A., and Maillard, J., Rev. Fr. Etud. Clin. Biol. **12**, 865 (1967).

4. Ovary, Z., Benacerraf, B., and Bloch, K. J., J. Exp. Med. **117**, 951 (1963).

5. Bloch, K. J., Kourilsky, F. M., Ovary, Z., and Benacerraf, B., J. Exp. Med. **117**, 965 (1963).

6. Spalter, J. S., and Ovary, Z., Proc. Soc. Exp. Biol. Med. **119**, 1073 (1965).

7. Bier, O. G., Passos, H. C., and Siqueira, M., Immunology **14**, 291 (1968).

8. Voisin, G. A., and Toullet, F., Ann. Inst. Pasteur, Paris **114**, 727 (1968).

9. Voisin, G. A., and Toullet, F., Ann. Inst. Pasteur, Paris **111**, 377 (1966).

10. Benacerraf, B., and Kabat, E. A., J. Immunol. **64**, 1 (1950).

11. Maillard, J. L., and Zarco, R. M., Ann. Inst. Pasteur, Paris **114**, 756 (1968).

12. Cochrane, C. G., J. Exp. Med. **118**, 489 (1963).

13. Stetson, C. A., J. Exp. Med. **94**, 347 (1951).

Received Sept. 8, 1969. P.S.E.B.M., 1970, Vol. 133.