ethanol, or roughly half his daily caloric requirement. Rats may not be satisfactory animals for studies of ethanol tolerance because of the extreme difficulty in achieving dosages similar to those of which man is capable. In order to give a dose equivalent to one half of a rat's requirement of 84 calories(10), it would be necessary to force it to metabolize 60 ml of 10% ethanol daily.

Such a consideration of dosage gives us little reason to expect a striking change in our rats, although they did receive a considerable amount of ethanol for an extended period of time. They did show an increase in rate approximately as large as their day to day variation, but we cannot conclude that this is evidence for an increased rate of elimination as the cause of the behavioral tolerance in humans. Species differences, differences in rats of administration and the nutritional effects of the various possible treatments are all variables which must be studied further.

Summary. Rates of $C^{14}O_2$ formation from ethanol-1-C^{14} were measured in rats that had been allowed only water, 20%, 10%, or 5% solutions of ethanol to drink. Rats which had drunk 10% ethanol for 6 to 11 months metabolized it 18% faster than controls or animals drinking 5% ethanol. Rats drinking 20% ethanol were not different from controls. None of the groups forced to drink ethanol differed significantly from controls in rate of $C^{14}O_2$ formation from acetate-1-C^{14} and glucose-U-C^{14}.

1. Hawkins, R. D., Kalant, H., Khanna, J. M., Canad. J. Physiol. Pharmacol., 1966, v44, 241.

2. Tolbert, B. M., Hughes, A. M., Kirk, M. R., Calvin, M., Arch. Biochem. Biophys., 1956, v60, 301.

3. Robinson, J. R., Chefurka, W., Anal. Biochem. 1964, v9, 197.

4. Von Wartburg, V. J. P., Rothlisberger, M., Helv. Physiol. Acta, 1961, v19 30.

5. Campos, I., Solodkowska, W., Munoz, E., Segovia-Riquelme, N., Cembrano, J., Mardones, J., Quart. J. Stud. Alc., 1964, v25, 417.

6. Greenberger, N. J., Cohen, R. B., Isselbacher, K. J., Lab. Invest., 1965, v14, 264.

7. Segovia-Riquelme, N., Campos, I., Solodkowska, W., Gonzales, G., Alvarado, R., Mardones, J., J. Biol. Chem., 1962, v237, 2038.

8. Marshall, E. K., Jr., Fritz, W. F., J. Pharm. Exp. Ther., 1943, v109, 431.

9. Nelson, G. H., Kinard, F. W., Quart. J. Stud. Alc., 1959, v20, 1.

10. Nutritional Requirements of Laboratory Animals, Publication 990, 1962, Nat. Acad. Sci. — Nat. Research Council, p53.

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Evidence of a Common Antigenic Specificity on Heavy Chains from Human IgG, IgM and IgA.* (32452)

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In recent years it has been possible to classify human immunoglobulins into classes and subclasses according to their antigenic specificities(1). The subclasses are often related to genetically controlled factors present on the molecules(2). Although κ and λ types‡ of light chains were early recognized as distinct in that they did not share antigenic specificity or fingerprint spots (4), examination of their amino acid sequences has shown similarities(5,6).

It has been believed for some time that the immunological cross-reactions between the human immunoglobulins IgG, IgM and IgA were dependent on common light chains only and that the heavy chains of these molecules

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‡ The immunoglobulin molecules and fragments are named following the system recommended by a committee of the World Health Organization (3).

were completely distinct. The finding of the H chain allotypic markers on the IgG(7) IgM (8,9) and IgA(10,11) of rabbit immunoglobulins demonstrated common antigenic groupings on the heavy chains of the rabbit and suggested that a similar situation would be found in other animal species. The research reported here provides evidence for such common antigenic specificities on the heavy chains of human immunoglobulins IgG, IgM, and IgA.

Methods and materials. IgG, IgM, and IgA paraproteins were purified using starch block electrophoresis, DEAE chromatography, and Sephadex filtration following initial precipitation with ammonium sulfate. These paraproteins were typed for their L chain subgroups using rabbit antisera specific for the κ and the λ types. Antisera were prepared in rabbits against the isolated paraproteins of both the κ and λ classes and against the isolated chains and papain subunits prepared from them. Repeated monthly injections of antigen in Freund's complete adjuvant were used to insure hyperimmunization. Purity of the injected antigens was assessed by immunoelectrophoresis, cellulose acetate and starch gel electrophoresis, and ultracentrifugation. The interactions of the antisera thus produced with appropriate paraproteins were examined by immunodiffusion techniques.

Results. Table I summarizes the reactions by gel diffusion against isolated Bence Jones proteins of 3 of the antisera used in further studies. The antiserum against Fab from and IgG (κ) paraprotein reacted with the η but not with the λ Bence Jones proteins. The reaction with κ Bence Jones protein of the anti-

TABLE I. Reaction of Antisera with Bence Jones Proteins.

Bence Jones proteins	Anti-Fab* No. 363	Anti-μ chain† No. 359
Banks (κ)	+	+
Hein. (κ)	+	+
Wilt. (λ)	0	0
Mont. (λ)	0	0
Will. (λ)	0	0

* Serum raised against Fab obtained from a κ IgG paraprotein, Ketch.

† Serum raised against μ chain obtained from a κ IgM paraprotein, Bart.

TABLE II. Reaction of Antisera with Paraproteins and Subunits.

Antigen*	Anti-IgG† absorbed with L chain No. 131	Anti-Fab‡ absorbed with normal Fc No. 363	Anti-μ chain§ No. 359
Fc (κ) Ketch.	+	0	0
Fc (κ, λ)	+	0	0
Fab (κ) Ketch.	+	+	+
L (κ)	0	+	+
L (λ)	0	0	0
IgG (λ) Will.	+	+	+
IgA (λ) Mont.	+	+	+
μ-chain (κ) Bart.	+	0	+
IgM (κ) Bart.	+	0	+

* All except the Fc (κ, λ) antigen were paraproteins or prepared from paraproteins. The L chain type of the paraprotein used is indicated in parentheses.

† Antiserum prepared against normal human IgG and absorbed with L chains prepared from normal IgG.

‡ Serum raised against Fab obtained from a κ IgG paraprotein, Ketch.

§ Serum raised against μ chain obtained from a κ IgM paraprotein, Bart.

serum raised against μ chain from the η IgM paraprotein is attributed to the presence of residual κ chain in the μ chain provoking antigen. It is known that approximately 10% of the L chain is very difficult to remove from rabbit IgG γ chain (12).

Table II summarizes the reactions by gel diffusion of these antisera and an antiserum against normal IgG with various paraproteins and subunits. The antiserum against normal IgG absorbed with normal L chain reacted with neither κ nor λ L chain, but did react with IgA and IgM paraprotein as well as with the μ chain prepared from the IgM paraprotein. Our thesis is that these reactions are due to an antigenic specificity common to the 3 different H chains characterizing the IgG, IgA, and IgM classes.

Antiserum to Fab from a κ IgG paraprotein and absorbed with normal Fc reacted with an IgA λ paraprotein, but contrary to expectations did not react with an IgM κ paraprotein or its μ chain.

Similarly antiserum prepared against μ chain from a κ IgM paraprotein reacted with a λ IgG paraprotein but not with Fc fragment prepared from either a normal IgG or a κ IgG paraprotein. Further evidence that this antiserum contained antibodies directed

against an H chain determinant common to the three classes of immunoglobulin was provided by the finding that this serum reacted with IgA paraprotein.

An antiserum prepared against intact Bart. IgM (not tabulated) failed to show cross-reaction, while that against the Bart. IgM μ chains did show the cross-reaction. For this reason, attempts were made to show some antigenic sites on the μ chain not accessible in the intact IgM molecule by absorption with pure IgM. It was found that such treatment removed all antibody activity from the anti-μ chain serum. Furthermore it was never possible to demonstrate formation of a spur of precipitation of the μ chain reaction over the whole IgM reaction.

Fig. 1 shows some double diffusion reac-

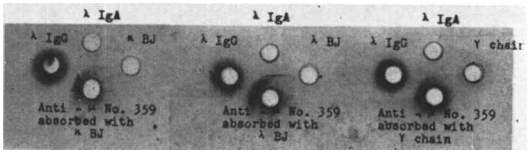

FIG. 1. Reaction of anti-μ chain with paraproteins. Concentration of IgG and IgA, 2 mg/ml; Bence Jones protein, 0.5 mg/ml; and γ-chain, 1 mg/ml.

tions employing antiserum to μ chain from IgM (κ) Bart. This slide shows that the line of precipitation formed with IgG (λ) Will. and the IgA (λ) Mont. was not removed by absorption of this antiserum with κ or λ type Bence Jones protein. However, absorption with Ketch. γ chain removed the reactivity completely. The slight line seen in the third pattern of Fig. 1 was visible only on the stained slide and subsequent addition of more heavy chain removed the reactivity completely.

Discussion. The double diffusion experiments indicate a common antigenic portion on the heavy chains of human IgG, IgA, and IgM. The major difficulty in interpretation of the reactions lies in the degree of certainty that no contamination is present in either the antigen or antiserum being studied. We believe that the evidence presented precludes such a simple explanation for the cross-reactivity observed.

The anti-μ chain serum No. 359 was prepared using as a provoking antigen μ chain prepared from a κ IgM paraprotein. In double diffusion experiments this protein at a concentration of 2 mg/ml showed no reactivity against λ antiserum. Since this technique can readily detect protein at 0.05 mg/ml, a contamination of less than 2% is indicated at this point. The antiserum provoked by immunization using μ chain prepared from this material did not react with λ chain (Table II). This most rigorous test of purity, use of the protein as an immunogen, failed to give evidence of contamination. The anti-μ chain serum No. 359 did not react with Fc fragment prepared from either the IgG paraprotein Ketch. or normal IgG. The recognized high antigenicity of the Fc fragment renders this absence of reaction particularly strong evidence against IgG contamination in the provoking antigen. Since serum No. 359 reacted with a λ IgG and a λ IgA paraprotein despite its inability to react with either λ chain or Fc from IgG (Fig. 1) it is logical to ascribe this cross-reactivity to a shared antigenic determinant on the Fd region of the γ, α and μ chains. The fact that the antibodies responsible for this reactivity are removed by absorption with λ chain supports this conclusion and excludes the possibility that the observed cross-reactivity could be attributed to contamination of the IgG and IgA paraproteins with IgM.

When purified human paraproteins are used to immunize rabbits the resulting antiserum may comprise a large number of specificities. Among these can be mentioned heavy and light chain subgroup activity(1), anti-Gm and anti-Inv factor activity(13), and some activity against an individual antigenic specificity of the molecules(14). To this list may be added reactivity related to a common antigenic specificity shared by the H chains of IgG, IgM, and IgA.

This common specificity appears to be relatively inaccessible in the whole molecule, and for this reason is not revealed by all antisera which might otherwise be expected to react with it. Particularly notable was one case in which the antiserum to the heavy chain showed the cross-reactivity while that to the intact molecule did not. Presumably removal of the L chains made the antigenic

site on the H chains more potent as a provoking antigen.

Studies with allotypic specificities have already provided evidence of a common antigenic grouping on rabbit immunoglobulins. The allotypic specificities of locus a, which had previously been shown to be present on the heavy chains of rabbit IgG were demonstrated to be present also on IgM(8,9) and IgA(10,11). Nussenzweig and Benacerraf (15) have shown that common antigenic specificities are present on the Fd fragments of guinea pig γ_1 and γ_2 immunoglobulins. Seligmann *et al*(16) have recently demonstrated similar cross-reactivity between human IgG and IgM. However, this antigenic determinant would appear to be unlike that being reported here in that it was dependent on the presence of intact and combined heavy and light chains. Similar relationship between protein conformation and antigenic specificity in human gamma globulins has been demonstrated for the Gm 3 factor(17).

Two other reports have come to our attention which indicate similar findings. Harboe and Deverill(18) have demonstrated similar cross-reactivity between IgG and IgM molecules and Kunkel *et al*(19) have found this cross-reactivity to encompass the IgA class. These authors also point to differences in behavior shown by various antisera and preparations of paraproteins, just as was encountered in the present experiments.

It would appear therefore that the immunologlobulins share, in addition to light chains, some antigenically similar, if not identical, portions on their respective heavy chains. The Fd portion of the γ chains is known to contain a variable portion(20), considered to be responsible for the antibody combining specificity, and presumably some constant portion(21). It is not possible to state the precise location of the antigenic determinants common to the heavy chains, although it would seem more likely to be the constant area for the specificities studied here, whereas the results of Seligmann *et al* (16) are suggestive that the variable sequence is involved.

The presence of a common antigenic specificity could be the reflection of a residuum in the evolution of the genes for the γ and α chains from the μ chain precursor(22). Such a view would be compatible with the data provided here. The findings in the rabbit with allelic specificities (allotypes) suggest that this simple explanation may not suffice(23).

Summary. Evidence is presented for a common antigenic specificity on the Fd region of human IgG, IgA, and IgM. The relationship of this finding to the evolution of immunoglobulin classes is briefly discussed.

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1. Kunkel, H. G., Harvey Lectures, 1965, v59, 219.
2. Martensson, L., Vox Sang., 1966, v11, 521.
3. Ceppellini, R., *et al,* Bull. Wld. Hlth. Org., 1964, v30, 447.
4. Putnam, F. W., Migita, S., Easley, C. W., in H. Peeters, Protides of the Biological Fluids, Elsevier, Amsterdam, 1963, p93.
5. Milstein, C., J. Mol. Biol., 1966, v21, 203.
6. Titani, K., Wikler, M., Putnam, F. W., Science, 1967, v155, 828.
7. Stemke, G. W., ibid., 1964, v145, 403.
8. Todd, C. W., Biochem. Biophys. Res. Comm., 1963, v11, 170.
9. Stemke, G. W., Fischer, R. J., Science, 1965, v150, 1298.
10. Feinstein, A., Nature (London), 1963, v199, 1197.
11. Sell, S., Immunochem., 1967, v4, 49.
12. Stemke, G. W., Science, 1964, v145, 403.
13. Litwin, S. D., Kunkel, H. G., Transfusion, 1966, v6, 140.
14. Slater, R. V., Ward, S. M., Kunkel, H. G., J. Exp. Med., 1955, v101, 85.
15. Nussenzweig, V., Benacerraf, B., J. Immunol., 1966, v97, 171.
16. Seligmann, M., Mihaesco, C., Meshaka, G., Science, 1966, v154, 790.
17. Polmar, S., Steinberg, A. G., ibid., 1964, v145, 928.
18. Harboe, M., Deverill, J., Acta Med. Scand., 1966, v179 Suppl., 74.
19. Kunkel, H. G., Grey, H. M., Solomon, A., Immunopathology, IV International Symposium, Grabar, P. & Miescher, P. A., ed., Grune & Stratton, Inc., New York, 1966, 220.
20. Press, E. M., Givol, D., Piggot, P. J., Porter, R. R., Wilkinson, J. M., Proc. Roy. Soc., B., 1966, v166, 150.
21. Pink, J. R. L., Milstein, C., Nature (London), 1967, v214, 92.
22. Singer, S. J., Doolittle, R. F., Science, 1966, v153, 13.

23. Todd, C. W., Inman, F. P., Immunochem., in press.

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Effect of Interaction of RNA and Polyglucose on Their Solubility in Water-Alcohol Mixtures.* (32453)

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Extraction of tissue with a mixture of phenol, deoxycholate and NaCl yields nucleic acids (DNA and RNA) which differ in biologic activity and physical properties from other preparations(1). The nucleic acid obtained by this method from viral infected tissue is infectious and unlike other preparations(2) the RNA is less soluble than DNA. The RNA is precipitated from aqueous salt solutions by 20% ethanol while 50% is required for DNA(3). The RNA so obtained by alcohol precipitation of the aqueous phase of the extraction contains much polyglucose (CHO) of 2-4 $\times$ 10^6 molecular weight(4). In view of reports elsewhere that the infectivity of nucleic acid isolated from poliovirus(5), gastroenteritis virus(6), or simian virus 40(7) is markedly enhanced by the presence of polysaccharide, it was considered possible that both the biologic activity and unusual solubility of RNA might not result entirely from its intrinsic properties but as a consequence of its interaction in solution with the CHO which accompanies it upon isolation. In the present study, experiments were designed to determine whether such interaction does occur with a resulting alteration in the physical properties of the CHO and RNA.

Methods. HeLa cells were grown in monolayers in Roux bottles with Eagle's medium (8). After removal of the growth medium and rinsing of the cultures with phosphate buffered saline (pH 7.2) 10 ml of 1 M NaCl containing 10% deoxycholate were added to remove the cells from the glass. The bottles were next rinsed with 10 ml of water-saturated phenol which was then combined with the 1 M NaCl. The mixture was shaken mechanically at room temperature. After separation of the aqueous phase, a second extraction with phenol was performed. The dissolved phenol was removed from the aqueous phase by ether extraction and the remaining ether removed by a stream of nitrogen gas. The procedure is essentially as described by Colter *et al*(1) for extraction of nucleic acids. The resulting aqueous phase contained DNA, RNA and CHO (polyglucose). The RNA and CHO were precipitated together free of DNA from the molar salt solution with 20% ethanol at 4°C by standing overnight, and then dissolved in 1 M NaCl(3).

The RNA content was determined by the orcinol method(9) and the CHO with a diphenylamine reagent(10,11).

Experimental and results. The RNA and CHO in a 1 M NaCl solution (prepared as described under *Methods* were separated by careful acid precipitation of the RNA. The two components were separately redissolved. A reconstituted mixture of the two was also prepared and the 3 samples, after being adjusted to equivalent concentrations in 1 M NaCl, were compared with a reprecipitated aliquot of the original mixture in regard to the solubility in 20% ethanol of the RNA and CHO constituents. Details of the procedure are as follows:

Sufficient 40% trichloroacetic acid (TCA) was added to 2 ml of a 1 M NaCl solution containing RNA and CHO (described under *Methods*) to give a final concentration of 5%. The resulting precipitate was washed

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