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Report on the Discussion of the Third Session

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PRESSMAN (*Roswell Park Memorial Institute*): I would like to ask all the speakers about their experience with antibody production in individual animals. After administration of the same antigen, individual animals can produce antiserum with somewhat different selectivities towards the antigen. Do the speakers routinely use pooled sera from groups of animals or serum from individual animals?

LEVINE: We never pool sera and, furthermore, we only use one animal, *i.e.*, guinea pig or rabbit, in one experiment for one antigen. Because of the expense of monkeys and the nature of our radioimmune assay experiments, we can use these animals over-and-over again. There is considerable variation with respect to the specificity of the antibodies produced to the prostaglandins (PG). We select the particular sera that will best suit our experimental needs.

ERLANGER: I second the comments of Dr. Levine. I will say that though we do get varying results from one animal to another, the antibodies to the ribonucleosides exhibit a remarkably high specificity toward these haptens. The degree of specificity is similar to that found by Dr. Steiner with antibodies to cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). If I can be a bit philosophical, the recognition systems in nature, with respect to purines and pyrimidines, are quite unambiguous despite the small structural differences between these substances. Apparently, nature can see some very distinct differences. With the steroids, on the other hand, one sees a lot of cross reactions as would be expected on the basis of similarity in structure. Apparently, this is the reason why purines and pyrimidines were chosen by nature for its message rather than something like steroids.

SPECTOR: Dr. Steiner, do you have comments along these lines?

STEINER: I would just like to emphasize that with respect to production of antibodies to the cyclic nucleotides, it is important to monitor continuously the specificity of the antisera after every booster injection, particularly with regard to adenosine triphosphate (ATP) recognition. Once we have antisera that is sensitive and does not bind very much ATP, then we have good antibody.

SPECTOR: Dr. Steiner, if you continue to immunize your animals, does the specificity of the antisera towards nucleotides begin to broaden?

STEINER: In general, the specificity does not change very much.

SPECTOR: In that regard, let me ask the panel a question. In relation to this problem of heterogeneity, one uses a protein as the carrier. I wonder if one might obtain a more homogeneous antibody population if a defined polypeptide were employed instead. Does the latter offer any advantage as a carrier?

LEVINE: In all the work I spoke about today, the carrier was polylysine. We have, however, used hemocyanin and albumin as carriers; all of them are suitable in producing antibody. With polylysine as carrier, the antibody response comes up but it does not increase as one keeps on boosting with antigen. With serum albumin or hemocyanin as carriers, the antibody response comes up more slowly but it reaches higher levels and continues to increase with booster doses.

ERLANGER: In our laboratory, we have used only protein as carriers. It is of note that you can use *homologous* protein, *e.g.*, rabbit conjugates, to immunize rabbits. In most cases, antibodies are produced but generally speaking, the titers are not as high in

the period of time that we use to immunize the rabbits as you would obtain with a conjugate of a heterologous protein.

(?) (*Hospital for Joint Diseases*): I have two questions for Dr. Levine and Dr. Spector. Dr. Levine, I would like to know whether you have measured the prostaglandins in any other tumor besides the bone resorptive fibrosarcoma?

LEVINE: I have looked in sera, not in tumors, of several hundreds of cancer patients. Only in one patient, and I am not sure about that yet, have we found any increase in prostaglandins.

(?) (*Hospital for Joint Diseases*): Dr. Spector do you feel that it is feasible to immunize an addict with the conjugate to heroin?

SPECTOR: We have not performed such experiments although Dr. Grace at the Naval Hospital has attempted to do that type of experiment with chimps. Dr. Grace, would you care to comment?

GRACE (*Naval Hospital*): Yes, we have been making a conjugate. We find that if with the 3-carboxymethyl morphine-serum albumin conjugate, antibodies are raised to heroin, morphine, and to some of the other related drugs but the antibodies are not of the reaginic type. They do not cause any harm. The animals, when challenged with heroin, do not go into anaphylaxis. However, we have been using as a carrier a roundworm extract which introduces a rather controlled immediate hypersensitivity response, *i.e.*, itching, hives, sort of an immunological antabuse. We are working on this as another aid in the armamentarium against heroin abuse.

ADLER (*New York*): I would like to make a comment on the presentation by Dr. Spector and raise a question or two. On the basis of our own studies, I concur with Dr. Spector's conclusion that the immunization against morphine affects some of the biological responses to the drug. My concern is about some of the data Dr. Spector has presented in support of this conclusion. I would like to ask the following questions. How much antibody did the immune mice possess at the time of the challenge to the analgesic dose of morphine? Secondly, what proportion of the 15 to 30 μg of morphine, which was the analgesic dose, was bound or neutralized or both by this amount of antibody? Thirdly, have you repeated and confirmed your data by using known amounts of passively-administered antibody to your mice? Lastly, I would like to comment on the question raised by Dr. Pressman on the differences of antibody responses by animals. Here, we unfortunately disagree with the findings by Dr. Spector and colleagues who indicated that antibodies raised against the carboxymethyl morphine-bovine serum albumin conjugate react poorly or not at all against the glucuronide of morphine. In studies on about 20 rabbits, we found that marked differences exist between the animals and furthermore differences are seen between bleedings from the same animal. Some sera recognize the bound morphine with 100% the efficiency of free morphine whereas other sera are detected with only 3% of the efficiency with which they react with free morphine.

SPECTOR: I do not know where to begin. Let me say, that we have taken samples from individual rabbits and have found variation from animal to animal even with those that are on carboxymethyl morphine in regard to their ability to bind the glucuronide. A number of the animals immunized with the carboxymethyl derivative do not bind the monoglucuronide as effectively as those that received the hemisuccinate derivative. In regard to the question on the immunization process, we have not performed the experiment you have posed, *i.e.*, passive immunization. This is a direction we have to take. In regard to the ability to bind labeled morphine, we find the same phenomenon reported on by Dr. Butler yesterday. With time, the amount of label sequestered out of the circulation by the antibody is increasing. We do not know what this means at present. We are not sure how much of that circulating morphine that we are administering (granted

that it is an impressive amount, 0.5 mg/kg.) must be sequestered out of the circulation and thus presented from getting to the critical site where the analgesic effect is elicited. As I pointed out in the talk, we have not actually measured the amount of morphine that exists in the central nervous system (CNS)—that is a critical point.

MARSELLIS (*Stony Brook*): This question is addressed to Dr. Levine. There have been a number of reports that in the nephrotic syndrome indomethacin sometimes has a remarkable effect on the proteinuria. Does this suggest that indomethacin might be working on a prostaglandin system? Do you know if prostaglandins have anything to do with protein transport in the kidney? We realize that these agents have a good deal to do with sodium transport.

LEVINE: I do not think I can answer these questions. I am not familiar with the work. It has been reported that some of the prostaglandins have effects in hypertension. With Dr. Rosenberg of the Boston City Hospital we have looked at many patients with essential hypertension as to the serum levels of PGA and PGB and found no differences from normal.

PRESSMAN: I would like to return to Dr. Spector's question concerning heterogeneity of antibodies in individual animals. There has been quite a bit of work with the synthetic carriers on the basis that the response to a more simple structure would be simpler. However, even with a complex carrier such as bovine γ-globulin which is made up of a good many globulin molecules, when a simple hapten is coupled the response of individual animals is to give a response of limited heterogeneity. Each animal produces primarily just a few types of antibody molecules which will react with that particular hapten group and these types of antibody molecules are different for each individual animal. This phenomenon has been extremely helpful in our structural studies and is of great help in the development of analytical reagents as described today. In this connection, I wish to ask Dr. Steiner if he thinks the same molecule of antibody reacts with both ATP and cAMP or if he has tried to purify his antibody by solid phase absorption to separate the ATP-interfering property.

STEINER: We have not performed those specific studies.

SPECTOR: If I may take the chairman's prerogative, I wish to ask Dr. Erlanger a question. He indicated in his talk that there might be a critical number of haptens attached to the carrier (approx. 10) for proper antibody production. Is the reverse true? Can you oversubstitute and thus prevent identification of the haptenic group?

ERLANGER: I cannot recall the exact paper but there has been a study dealing with this question. There was an optimum number of groups—too many groups can cut down the response.

(?) (*Jefferson Medical College*): In recent months, we have been looking at a number of haptens and comparing the responses when coupled to polypeptides or to proteins. We have not found any difference, except in one case, in either the intensity or specificity of the antibody response. Secondly, we have been interested in looking at antibodies to the morpholine ring and, depending upon the route of administration of antigen, this is one of the most potent haptens ever reported.

ERLANGER: Could you be a bit more specific about the nature of the morpholine group?

(?) (*Jefferson Medical College*): We are using polyglutamic acid as the carrier in which a morpholino-ethylamine group is covalently attached to the *gamma* carboxyl-groups. The specificity is directed towards the morpholino ring.

GILLETTE (*N. I. H.*): I have three unrelated questions. When you slow down the half-life of morphine, have you ever challenged with a cold dose of drug to see whether you could displace the morphine from the antibody *in vivo*?

SPECTOR: No, we have not.

GILLETTE: When you use the serum al-

bumin from the same species in which you are inducing antibody formation, do you require more haptenic groups?

LEVINE: No, we do not see that requirement.

GILLETTE: Has anyone attempted to obtain an inbred strain of animal in which the antibody response would be uniform?

Panel: No.

STERN (*Baxter Labs.*): One of the possibilities which has not been indicated to this point is that you can turn the radioimmune assay around and have now an exquisitely sensitive method for antibody development toward the material you are looking at. Is it possible that the long life of morphine in some of the patients was due to the development of antibody to the drug?

SPECTOR: That question has arisen a number of times, *i.e.*, are there endogenous levels of antibody in individuals who exhibit a tolerance to a specific drug. One might look at a population of addicts to test this possibility. In a small population of addicts we were unable to sustain this hypothesis. Parker and Williams had published a report about some patients who did have antibodies.

LEVINE: May I add something to that. We have done an exhaustive study with Dr. Richter of Harlem Hospital to look for antibodies to heroin in selected patients. We pushed the study since we felt that any antibody present would probably be a weakly-binding one. We were still unable to obtain a clear cut answer to this problem even with the use of a multivalent antigen.

BLAKE (*University of Maryland*): Dr. Spector, have you considered that the apparent reduction in analgesic potency to morphine could be caused by the loss of the morphine conjugate from the immunogen resulting in a type of pharmacological tolerance.

SPECTOR: The amount of morphine on the immunogen is so small that one would not anticipate the development of tolerance by its release. In addition, we used the procedure employed by Dr. Way (University of California) to ascertain tolerance or addiction—we were unable to detect any.

ERLANGER: I would like to ask Dr. Levine a question. When you immunize with PGE, you get antibodies that react with PGB. I was wondering whether the PGE levels in rabbits were very high and perhaps you were selecting out for the cross reaction with PGB.

LEVINE: I had not thought about it in that way. In fact, PGE levels are high. Other workers, however, have made antibodies directed toward PGE. Their synthesis of the PGE-conjugate is different and, furthermore, the conjugate is used fresh. These differences may be sufficient to explain the results of the different labs.

SPECTOR: I would like to take this opportunity to thank the speakers and the audience for their participation.