dependent on the rate of rise of the voltage impressed between the subject and the electrode, and the rate of rise is governed by the applied voltage waveform and the voltage drop across the resistance. The range of streamers is proportional to the corona onset voltage. However, we have not seen any influence of large changes in skin resistance on streamer range. Presumably, this is due to the shunting effect of skin capacitance.

In general, the photographic response to moisture suggests that corona discharge photography may be useful in the detection and quantification of moisture in animate and inanimate specimens through the orderly modulation of the image due to various levels of moisture.

References and Notes

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Gammaflow: A Completely Automated Radioimmunoassay System

Results become available in minutes.

Gary Brooker, Wesley L. Terasaki, Michael G. Price

The monumental discovery of Yalow and Berson (*1*) of an isotope displacement method in which insulin-specific antibodies and radioactively labeled insulin are used to measure minute quantities of this hormone has led to the widespread application of this technique (termed radioimmunoassay) for the analysis of biochemically and clinically important substances. Because antibodies with ultrahigh selectivity and affinity can be obtained, measurements of virtually any desired compound in amounts as low as 1 femtomole (10^{-15} mole) can be made in rather impure samples. This advantage has made radioimmunoassay the technique of choice for all but the most easily detectable compounds. The reagents are easily obtained or prepared, and the antiserums are used at such a high dilution that 1 milliliter of a good antiserum can be sufficient for hundreds of thousands of assays.

A typical assay initially involves combination of unknown samples or standards with a specific isotope tracer (radioactively labeled ligand) and antibody. This solution is then incubated for minutes, hours, or even days to obtain equilibrium between the antigen (ligand molecule being measured) and the antibody. The antibody-bound labeled ligand is then separated from free labeled ligand. Separation is usually accomplished by treatment with dextran- or albumin-coated charcoal (which absorbs the free, unlabeled or labeled ligand), by precipitation of the complex of antibody and labeled ligand with ammonium sulfate or alcohol, or by some other technique, such as molecular sieve chromatography. The labeled ligand–antibody complex is recovered after centrifugation or by collection of a specific column fraction, and the radioactivity is determined in an automatic beta or gamma radiation counter. The amount of unknown substance present is determined by interpolation from standard curves constructed from standards measured at the same time. Increasing amounts of nonradioactive ligand reduce the specific activity of the labeled ligand thus yielding less radioactivity bound to the antibody.

The manual processing of samples for radioimmunoassay is time-consuming and requires meticulous attention to detail in order to obtain reproducible results. In our laboratory alone we use 8,000 to 10,000 test tubes per month for the radioimmunoassay of cyclic nucleotides. The repetitive and thus boring nature of the radioimmunoassay tech-

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nique makes it prone to human error. Attempts to completely automate radioimmunoassays have met with only limited success (*2*) because there have been difficulties in devising online methods to separate and measure free labeled ligand from that bound to the antibody.

We now report the details and initial results of a completely automated on-line radioimmunoassay system, "Gammaflow" (*3*), which combines the sample or standard with the labeled ligand and antibody, incubates the samples, separates and determines the amount of labeled ligand bound, and then computes the amount of the unknown sample by comparison to standard curves. The complete operation, at the rate of 20 to 40 samples per hour, can take place in the absence of any human intervention. To date we have performed automated radioimmunoassays for digoxin, cyclic adenosine monophosphate (cyclic AMP), cyclic guanosine monophosphate (cyclic GMP), insulin, angiotensin I, and thyroxine.

The System

The Gammaflow automated radioimmunoassay system consists of a peristaltic pump, a sample pickup device, a sodium iodide gamma-radiation detector, a column to separate free from bound radioactivity, associated miniature solenoid valves to control solution flow, control circuitry, and a minicomputer to store and calculate the results as they become available (Fig. 1). Despite the heavy workload of which this instrument is capable, it only occupies about 1 meter of bench space and requires no external support systems other than an electrical power source.

The Gammaflow system comprises a number of unique features aimed at (i) combining accurately measured volumes of sample, labeled ligand, and antiserum solutions, (ii) incubating these for a precise length of time under controlled temperature conditions, (iii) separating the free from bound ligand, (iv) counting the bound labeled ligand, and (v) making all necessary calculations for radioimmunoassay. The initial sampling and mixing of reagents is accomplished essentially by a continuous flow design that has proved itself with respect to accuracy, simplicity, and flexibility in many colorimetric procedures. The Gammaflow concept, however, departs from conventional flow systems in major ways. The valuable reactants are discretely introduced coincident with each sample rather than wasted between sample draws, as is typical of a continuous flow system. The radioactive sample (reaction mixture) itself triggers the preset timed sequence that controls the sample flow through the separating and counting portions of the system. Analysis time is decreased because the sample, once detected, is sped on its way by means of a high buffer flow mechanism. Separation of free from bound labeled ligand is accomplished by a versatile mixed-bed separating column usable for repeated analyses without loss of efficiency. Counting accuracy of the radioactive sample is maximized by a stop-flow technique which allows for stationary scaler counting of the separated bound labeled ligand and its subsequent rapid elimination from the detection system. Unique to Gammaflow is its ability to perform different radioimmunoassays on consecutive samples with no time loss between analyses.

The complete flow pattern of the system is shown in Fig. 2. The system is designed so that the sampler draws the solutions of sample, labeled ligand and antiserum once every 3 minutes for 30 seconds (*3a*). This then leaves a 2.5-minute space of buffer between samples and eliminates problems of sample diffusion and carry-over. Since each sample triggers its own processing sequence once it reaches the flow-through loop of the radiation detector, small deviations in flow rate or stoppage of the sampler to load more samples does not alter the constancy of time periods used for sample processing. However, it is important that the peristaltic pump not deviate in flow rate if all the assays are to have the same incubation time. Another important feature of the system is the use of an anion-exchange resin such as Dowex 1 to absorb free labeled ligand. Immunoglobulins are weakly charged molecules and pass freely through the resin, so such a resin can be used to separate unbound labeled ligands from those bound to their specific antibodies. Charcoal may be added to the column to serve as an additional absorbant. The columns have remarkable capacity and stability. We have at times run more than 650 samples containing various labeled ligands and serum samples on the same column without any appreciable change in background count.

The incubated sample is applied to the column as it emerges from the first passage through the radiation detector. The air space above the column is open to the atmosphere while the sample is applied (since valve 4 is open to the air). Thus the complete sample is applied to the column and the air bubbles are dispelled at the bed surface. The sample is thus quantitatively applied to the resin. The level of the liquid of the column is maintained at just that of the resin bed by the hydrostatic head generated by the difference in height between the top of the column and the position of the exit tube from valve 5 and by the hydration properties of the column material itself. Upon initiation of the high flow pattern (valves

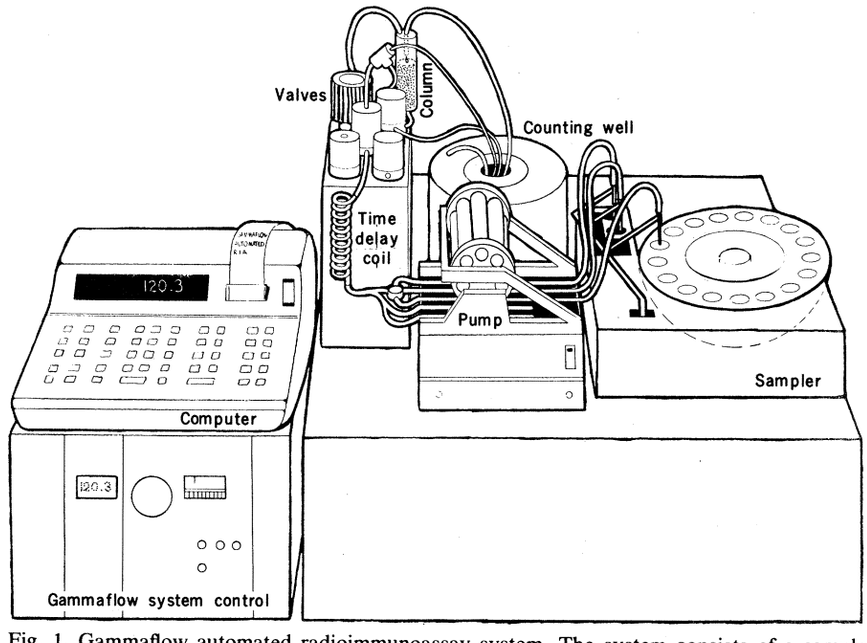

Fig. 1. Gammaflow automated radioimmunoassay system. The system consists of a sample pickup device, peristaltic pump, time delay coil, flow control valves, separation column, gamma radiation detector (NaI crystal scintillation detector), electronic control circuitry, and on-line minicomputer.

1, 2, 4, and 5 are then actuated) the antibody-bound labeled ligand is rapidly (about 20 seconds) forced through the resin and into a Teflon reservoir where scaler counting commences as soon as the chamber fills.

Sequence and Assay Calculation

The timing and control circuitry of the instrument has been hard-wired with integrated circuits: a single plug-in card containing a set of variable resistors controls the complete assay time sequence, and a different sample processing rate or time sequence can be easily obtained by changing the timing card. The Gammaflow system has been interfaced with a commercial minicomputer for data calcu-

Table 1. Reagents for the automated radioimmunoassay. Solution 1 was 50 m*M* sodium acetate, *p*H 4.75, containing 0.015 percent Brij-35; solution 2 was 50 m*M* tris-HCl, *p*H 9.2, containing 0.015 percent Brij-35. The thyroxine isotope solution also contained 1:5000 sodium Merthiolate. The isotopically labeled ligands (radioligands) were dissolved in either solution 1 or solution 2. Antiserums were diluted in the Buffer Line buffer. ScAMP-TME (2′-*O*-succinyl cyclic AMP tyrosine methyl ester) and ScGMP-TME (2′-*O*-succinyl cyclic GMP tyrosine methyl ester) were labeled with ^{125}I and purified (*6*). Specific activity was between 100 to 200 c/mmole. ^{125}I-labeled digoxigenin-3-*O*-tyrosine (2200 c/mmole) was a gift of Schwarz/Mann. Monoiodinated (^{125}I) insulin (100 μc/μg), monoiodinated (^{125}I) angiotensin I (687 μc/μg), ^{125}I-labeled thyroxine (118 μg/μg) and carrier-free Na^{125}I were purchased from New England Nuclear. Standards were gravimetrically prepared and diluted in either solution 1 or solution 2 (digoxin standards made in digoxin-free serum assayed identically to standards prepared in buffer). Angiotensin I was obtained from Calbiochem, porcine insulin from Novo Pharmaceuticals, and digoxin, cyclic AMP, cyclic GMP, and thyroxine from Sigma. Cyclic AMP and cyclic GMP standards were acetylated with acetic anhydride in aqueous solution as described (*6*). The column was poured simply as a 1:1 (by volume) slurry of charcoal and anion-exchange resin (AG 1-X8, 100 to 200 mesh).

Compound	[^{125}I]ligand (0.2 μc/ml)	Antiserum	Buffer solutions			Time delay coil	Temperature
			Buffer Line	High flow	Sampler wash		
Digoxin	Digoxigenin 3-*O*-Tyrosine	1:30,000	Solution 1 plus bovine albumin (5 mg/ml)	Solution 1 without Brij-35	Solution 1	3	Room
Cyclic AMP	ScAMP-TME	1:2,000				21	Room
Cyclic GMP	ScGMP-TME	1:1,000				21	Room
Insulin	Monoiodinated insulin	1:120,000	Solution 2	Solution 2 without Brij-35	Solution 2	21	39°C
Angiotensin I	Monoiodinated angiotensin I	1:2,500				21	Room
Thyroxine (T4)	Thyroxine	500-tube commercial antiserum in 50 ml				9	Room

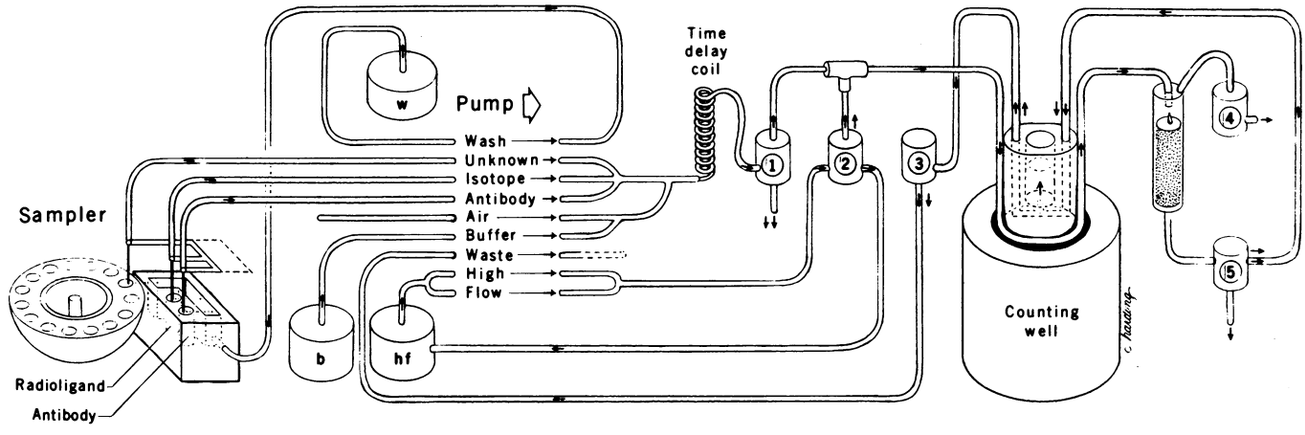

Fig. 2. Flow diagram of the Gammaflow automated radioimmunoassay process. Standards, unknown samples, the labeled ligand, and the antiserum solution are placed in the sampler. When the sample rate is 20 per hour, samples are sequentially drawn for 30 seconds every 3 minutes coincident with isotope and antibody pickup. The reaction ingredients mix as they emerge from the peristaltic pump (the tubing lengths were initially adjusted so that the reactants arrived at the point of mixing concurrently), and binding proceeds as the samples flow in the time delay coil for 3 to 21 minutes (depending upon the coil length). All radioimmunoassay incubations were performed at 23°C except for the insulin assay in which the coil was placed in an incubation bath at 39°C. The reaction mixture (as a discrete segment) was delivered from the incubation coil through a bypass or isolation valve (valve 1) and into a NaI γ-counting well, which detects the presence of radiation as it flows through a short loop. A preset, valve timing sequence is triggered then by the sample itself. Seventy-five seconds later, when virtually all the sample has passed through the gamma detector (for the first time) and has been applied to the separating column, valves 1, 2, 4, and 5 are actuated simultaneously. Valve 1 isolates the detection and separation system from the incubation coil and pump by bypassing the oncoming buffer (that is, the spacer between sample segments) to waste. When valve 2 is actuated, buffer is infused into the main line at a high (7.8 ml/min) flow rate, thus flushing the flow-through loop and eluting the separating column. Since valve 4 is now actuated, the system is closed and a high flow rate is attained in the column. The column eluate is directed by valve 5 into a specially constructed all-Teflon counting chamber; quantitative elution of antibody-bound ligand is achieved in 25 seconds at which time valve 5 switches, directing further column effluent to waste, and effectively stopping the separated sample in the counting chamber. The sample is scaler-counted for 50 seconds. Valves 1, 2, and 4 de-energize 15 seconds before the termination of the sample count, thereby reconnecting the sample stream and allowing the next incubated sample to approach the detection system. At the end of the 50-second sample count, valve 3 opens and the vacuum built up in the line quickly (2 seconds) evacuates the radioactivity from the Teflon counting cell. The sample counts are automatically entered into the system computer. The instrument is now ready to process the next sample and begins the sequence once again when triggered by the appearance of radioactivity from the next sample. The valve and counting times have been reduced for processing rates of up to 40 samples per hour. Flow rates are 2.5 ml/min for wash; 0.10 ml/min for unknown, isotope and antibody; 0.32 ml/min for air; 0.23 ml/min for buffer, 3.9 ml/min for waste; and 7.8 ml/min for the combined high flow tubes. The double arrows indicate the alternate flow path when each three-way valve is actuated.

lation and data output. The H-P 9815 (Hewlett-Packard) minicomputer is inexpensive and yet can store enough data to calculate standard curves by means of a routine which iteratively determines the best fit of the data. In one printout mode (the data handling is quite flexible and many other methods of calculation are possible) the previously programmed computer tells the operator the number of standards (along with their concentrations) to be loaded on the sampler. Usually a standard that does not contain the ligand being measured initiates the series. The standards and unknown samples are then loaded, and the instrument is started. Once the first standard is processed (the number of counts observed for it is termed B_0), the subsequent standards are processed and their respective counts are collected. The printout then shows the counts, the B/B_0 ratio, and the sample amount. As soon as the last standard has been processed, the computer begins a log-log linear regression fit of the data in which plots of the log of B/B_0 as a function of the log of total ligand (including labeled ligand) are compared. The labeled ligand amount is incrementally increased until the best fit of the data is obtained. The calculated amount giving the best fit and the correlation coefficient of the generated curve are then printed. This process takes 10 to 20 seconds. As the first unknown is processed the B/B_0 ratio is compared to the standard curve previously generated, and the calculated amount of ligand is interpolated from the standard curve.

Assays and Assay Conditions

Radioimmunoassays for six compounds of diverse structure have been accomplished to date by the Gammaflow automated radioimmunoassay system. These assays are insulin (*1*), digoxin (*4*), cyclic AMP (*5, 6*), angiotensin I (*7*), thyroxine (*8*), and cyclic GMP (*5, 6*). The specific assay buffers and conditions used are shown in Table 1. All of the labeled ligands were prepared by us according to published methods, or obtained from commercial sources and used without further purification. In fact, the presence of impurities such as ^{125}I-labeled iodide ion in the labeled ligand solutions is of no consequence since the Dowex column binds iodide ion very strongly. The diluted isotope and antiserum solutions remain stable for at least 1 day at room temperature. The cyclic AMP and cyclic GMP radioimmunoassays were performed at *p*H 4.75 since the 2′-*O*-acetylated derivatives are more stable at this *p*H. The digoxin assay was performed at this *p*H since the cyclic nucleotide assays were performed at the same time; however, it is likely that the digoxin assay could be easily performed at another *p*H. The *p*H 9.2 buffer selected for the thyroxine assay was similar to that reported for the manual thyrox-

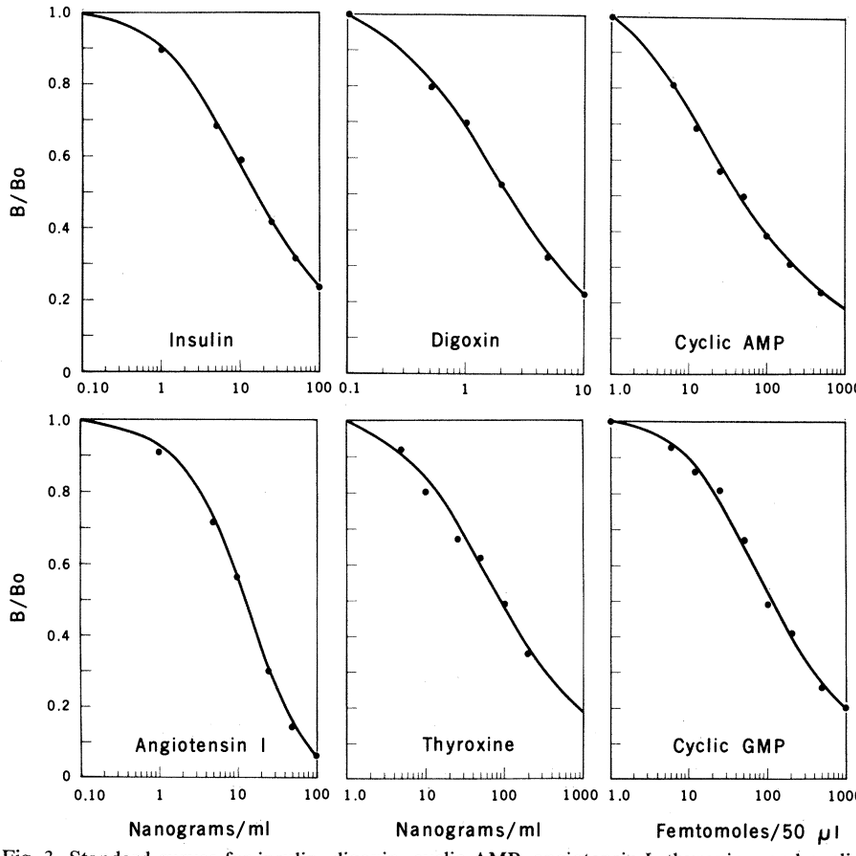

Fig. 3. Standard curves for insulin, digoxin, cyclic AMP, angiotensin I, thyroxine, and cyclic GMP. Assay conditions are shown in Table 1. Each point is the mean of three to eight determinations. Standard error bars were not included since they were about the size of each plotted point. The mean coefficient of variation of B/B_0 was 2.1 percent for all the standard curves. The coefficient of variation for each point varied between 1.7 and 5 percent depending on the count rate. The solid line is the iterative computer-fitted line. The value of B_0 (in counts) was 1990 for insulin, 3390 for digoxin, 3325 for cyclic AMP, 2290 for angiotensin I, 1540 for thyroxine, and 2153 for cyclic GMP; the regression coefficients of the lines were 0.999, 0.999, 0.998, 0.999, 0.985, and 0.997, respectively.

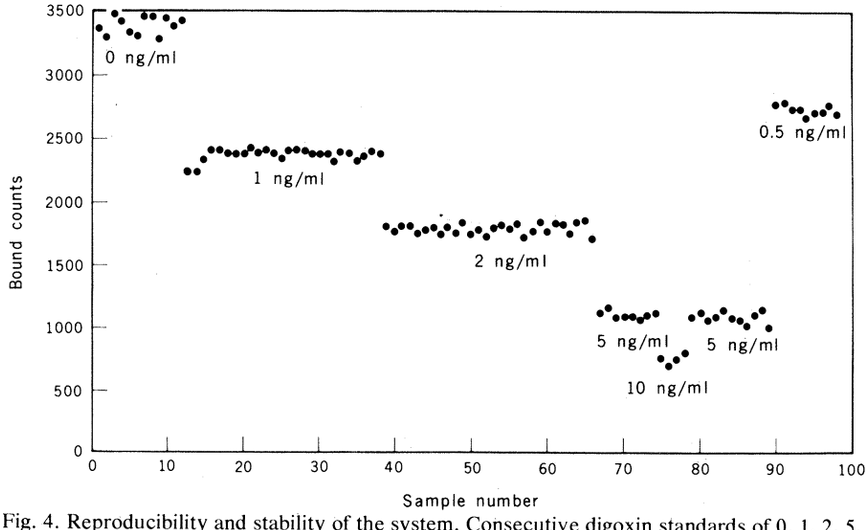

Fig. 4. Reproducibility and stability of the system. Consecutive digoxin standards of 0, 1, 2, 5, 10, and 0.5 ng/ml were processed. These 98 samples represent nearly 5 hours of instrument operation.

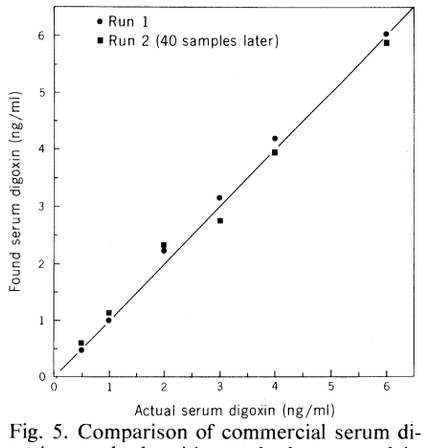

Fig. 5. Comparison of commercial serum digoxin standards with standards prepared in the laboratory. Commercial serum digoxin standards of 0.5, 1, 2, 3, 4, and 6 ng/ml obtained from the Burroughs-Wellcome Co. were assayed on the instrument. The instrument was first standardized with digoxin standards of 0.5, 1, 2, 5, and 10 ng/ml prepared with digoxin (Sigma). No significant instrument drift was seen when the standards were assayed 2 hours (40 samples) later.

ine radioimmunoassay (*9*). Insulin and angiotensin I were also assayed at this *p*H. Initial attempts to assay these peptides at *p*H 4.75 were unsuccessful since their respective labeled ligands appeared to adhere to the plastic tubes of the pump. These assays are typically performed by manual techniques at *p*H 7.5 or higher. The antiserums were diluted, as indicated, to bind 20 to 50 percent of the total labeled ligand.

Standard curves for insulin, digoxin, cyclic AMP, angiotensin I, thyroxine, and cyclic GMP are shown in Fig. 3. The slope of the curves and sensitivity of these assays are in most cases comparable to those obtained with the manual procedures. The slope of any radioimmunoassay curve is dependent on the binding constant of the antibody and the amount of labeled ligand used (*10*). The cyclic nucleotide assays shown here are very close in sensitivity and slope to our modifications (*6*) of the original radioimmunoassays for cyclic nucleotides (*5*). The assay for serum digoxin performed by the Gammaflow automated radioimmunoassay system is exquisitely sensitive and can easily detect the difference between toxic and nontoxic levels of digoxin in serum. Nontoxic levels are 1.4 ng/ml while toxic levels average 3.4 ng/ml (*4, 11*). Therefore, mean nontoxic levels would have B/B_0 equal to about 0.6 and toxic levels would yield B/B_0 values of 0.4.

The standard curve obtained for thyroxine is nearly superimposable with published manual thyroxine radioimmunoassay curves (*9, 12*). The standard curve for insulin has adequate sensitivity for most clinical applications and is similar in slope and sensitivity to the standard curve provided by the supplier of the isotope and antiserums. It is important to consider that it was obtained after a 21-minute incubation and that most insulin assays require 2 to 3 days of incubation (*13*). The assay of angiotensin I was readily accomplished with the Gammaflow system, with a sensitivity adequate to measure plasma renin activity. In general, the standard curves in Fig. 3 demonstrate the versatility of the system so that rapid radioimmunoassays can be performed in a completely automatic manner for a wide variety of substances. Successfully demonstrated here are radioimmunoassays for nucleotides; a steroidlike substance, digoxin; large and small peptides, insulin, and angiotensin I; and a small organic molecule, thyroxine. It seems probable that most compounds now measured by radioimmunoassay could be readily adapted to this system.

Table 2. Demonstration of the versatility of the system to sequentially perform radioimmunoassays for different compounds. The ratio B/B_0 was calculated from the first zero standard of the respective compound. The nine samples as indicated were placed in the sampler tray. The corresponding isotope and antiserum solutions were then placed in the sampler prior to the pickup of each sample. No delay between samples occurred and it took 27 minutes for all nine samples to be drawn into the instrument. Incubation time was 21 minutes at 39°C.

Sample No.	Compound	Amount (ng/ml)	B/B_0
1	Angiotensin I	0	1.00
2	Angiotensin I	25	0.23
3	Insulin	0	1.00
4	Thyroxine	0	1.00
5	Insulin	25	0.45
6	Thyroxine	0	1.05
7	Insulin	25	0.42
8	Angiotensin I	25	0.20
9	Insulin	0	0.95

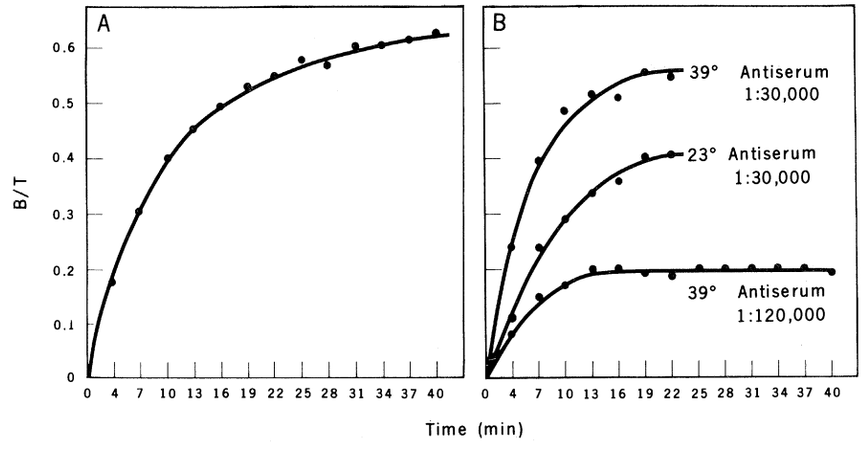

Fig. 6. Determination of antibody-radioligand binding time course with the Gammaflow automated radioimmunoassay system. Antiserum and labeled ligand were mixed (this is time zero) and placed in a temperature jacketed cup permanently positioned in place of the sampler wheel. A portion of the antigen-antibody solution was automatically drawn every 3 minutes for up to 40 minutes to generate the time points on the curves. The incubation line was shortened so that each sample was processed within 4 minutes after being drawn into the system. The antibody cup was replaced with buffer, and the isotope and sample probes combined to sample the mixture of labeled ligand and antibody. (A) Time course of binding of angiotensin I at room temperature. Antiserum and isotope as in Table 1. (B) Time course of binding of insulin at 23° and 39°C as a function of antiserum concentration. The initial rate of binding was increased at elevated temperature, and with diluted antiserum equilibrium was apparent after 13 minutes.

Reproducibility and Stability

An unattended radioimmunoassay system, if it is to be truly automatic, must have long-range stability and reproducibility. We made such checks on reproducibility, stability, and carry-over between samples when assaying for serum digoxin. Figure 4 shows the actual radioactivity bound for 98 consecutive digoxin standards. It is apparent from these data that the system is remarkably stable with little variation between replicates. A switch from one concentration to another is immediately followed by a new steady state. In another series of experiments, we alternated extremely high concentrations of digoxin (1000 ng/ml) with a zero standard in order to determine the amount of carry-over between samples, which we found to be less than 0.1 percent. Therefore, the interference of one sample upon the next is negligible. This

type of stability is observed in all of the assays performed with the present automated radioimmunoassay system.

Serum Digoxin

When 20 randomly selected serum samples were analyzed for digoxin only once by the automated radioimmunoassay, excellent and statistically significant correlation ($r = .75$, $P < .001$) between this method and the method used by our hospital (*14*) was obtained. It is important to point out that the assay of the 20 samples took only 1 hour and that the first results became available within 5 minutes. It seems probable that a determination of serum digoxin could be performed in less than 3 minutes if that speed were desired in an emergency situation. In contrast, the manual assay was started in the morning, and results were not available until the end of the day. There are clear cases where rapid turnaround, now possible with this automated radioimmunoassay system, could be of great benefit in the management of patients with suspected digitalis intoxication.

We also decided to test the automated radioimmunoassay system's linearity and stability by assaying standards of digoxin in serum. When these commercial standards were assayed at the beginning and at the end of a run, an excellent correlation was obtained when compared to the gravimetrically prepared standards used initially to standardize the system as shown in Fig. 5.

System Flexibility

The present Gammaflow automated radioimmunoassay system is especially well suited to establish new radioimmunoassay protocols rapidly since the basic reagents which vary between different radioimmunoassays (sample, labeled ligand, and antiserum) are discretely drawn into the system. The system therefore contains all of the advantages of the stability of continuous flow analysis and the flexibility inherent in discrete-sample systems. Because there is no carry-over between samples it is easy to perform alternate radioimmunoassays without any change in the system except to change the labeled ligand and antiserum solutions. In fact, it is even possible that the antiserums for various ligands could be combined, and only the sample and appropriate labeled ligand need be supplied. Table 2 shows alternate radioimmunoassays for angiotensin I, insulin, and thyroxine.

It is possible that certain radioimmunoassays may not be immediately adaptable to the Gammaflow system in the described configuration above because the specific rate of an antigen-antibody interaction is too slow for coil incubation or because the column material does not effectively or selectively bind the free antigen. Certain assays may require off-line incubation and that possibility is now under study. In addition, our system is limited in the sense that only compounds that are retained by the charcoal-Dowex column or similar materials could be assayed at present. While we have already shown that this means of separation works for a number of compounds, we anticipate that column materials of different compositions could be used in the development of methods in which charcoal-Dowex is ineffective.

Binding Time Courses

Optimal radioimmunoassay sensitivity and stability is best accomplished when the B_0/T is about 0.5 (where T is the total amount of radioactivity present during each incubation). In establishing new assays, it is useful to determine rapidly how long the samples need to incubate and what antiserum dilution is required to obtain optimal assay results. While elevation of temperature tends to reduce assay sensitivity for theoretical reasons which have been previously developed (*10, 15*), the ability to study the effects of temperature and time on the binding reaction permits optimization of these parameters. A considerable advantage of the Gammaflow system is its ability to perform radioimmunoassays at nonequilibrium conditions since the incubation time of the sample is constant from sample to sample. This capability of the instrument can be used to determine the time course of binding when the antiserum and labeled ligand are already mixed and repeatedly sampled. Figure 6A shows the automated determination of the time course of binding between angiotension I and its antibody, and Fig. 6B demonstrates the time course of insulin binding at various temperatures and antiserum dilutions. It is apparent that the reaction of angiotensin I and antibody begins to reach equilibrium rather rapidly at room temperature. The rate of association of insulin with the antibody is increased at elevated temperature as shown in Fig. 6B.

Conclusion

The Gammaflow automated radioimmunoassay system described here can be used for various radioimmunoassays with little or no change in the instrument and can even process different radioimmunoassay samples one after another. Therefore, it is possible that the analytical capabilities of many laboratories could be broadened and expanded by the use of this new instrumentation. The number of samples that can be processed per day by one Gammaflow instrument is probably greater than that possible by several technicians using a considerable amount of expensive equipment and supplies including centrifuges and automatic radiation counters. An important aspect of the Gammaflow system is that it offers a laboratory the flexibility of doing different radioimmunoassays faster than would be possible by other, often tedious methods.

The assays described here have not yet been optimized with respect to slope or sensitivity. While the sensitivity appears adequate for most purposes, even these assays could probably be improved in sensitivity, slope, or assay rate. The variables of the system include incubation time and temperature, number of samples per hour, antibody titer, concentration of labeled ligand, and specific activity. The sample size can even be altered by simply changing the size of the sample pump tube. The charcoal-Dowex column may not work for all radioimmunoassays; however, it has worked well as the means of separation in all assays that we have attempted. This possibility need not limit the potential use of the system. Other types of column separators, such as molecular sieves, could be used. Another approach would be to immobilize excesses of a specific antibody on a column support to act as a means of binding free labeled ligand that did not bind to the same soluble antibody during the initial incubation.

The complete automation of radioimmunoassay makes results of analyses available in minutes rather than in hours or days as compared to the use of conventional manual techniques.

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Picosecond Chemistry

A variety of ultrafast molecular processes have been measured by picosecond spectroscopic techniques.

G. E. Busch and P. M. Rentzepis

Intense light pulses with a duration of a few picoseconds (10^{-12} second) generated by mode-locked lasers were first utilized for studies of ultrafast physical and chemical processes (*1*) in 1967. Since then, there has been a tremendous growth in the number and variety of experiments being conducted by picosecond spectroscopic techniques. These experiments have led to more sensitive and refined data; for example, the 1967 7-psec value for the relaxation time of azulene had been reduced to 4 psec by 1974 (*2*). Although a large number of research groups are actively engaged in investigations of ultrafast phenomena by picosecond methods, only a small number are directly involved in measuring chemical interactions.

A partial list of general research areas in chemistry in which picosecond spectroscopy has been used to probe ultrafast molecular processes includes: (i) vibrational relaxation within the ground electronic states of small molecules and within the excited states of large molecules; (ii) rates of internal conversion between singlet molecular electronic states and of intersystem crossing between singlet and triplet states; (iii) orientational relaxation rates of large molecules in solution; (iv) the dynamics of the trapping and solvation of excess electrons; (v) fast relaxation processes in excited laser dye molecules; (vi) cage effects on chemical reactions in solution; and (vii) primary events in vision and photosynthesis. Several investigators (*3*) have reviewed the work that had been done in these and other areas of ultrafast measurements up to 1974. We will not attempt here to present a comprehensive treatment of all the research that has been conducted since that time, but instead we will discuss progress in several areas that have been of particular interest to us. We begin with a brief description of some experimental techniques commonly used in picosecond studies.

Experimental Method

Numerous variations in experimental arrangements can be used to acquire data on ultrafast processes (*2–4*). The choice of a suitable configuration depends as much on individual preference as on the wavelength, intensity, and resolution requirements imposed by a particular experiment. A typical time-resolved picosecond spectroscopy experiment consists of (i) the irradiation of a sample with a laser pulse having a duration of a few picoseconds, (ii) probing of the sample before, during, and after excitation, and (iii) acquisition and analysis of experimental data. A general experimental configuration used to carry out these operations is illustrated schematically in Fig. 1. In the majority of our experiments, the laser system consists of a neodymium-doped glass oscillator and amplifier. The oscillator produces a train of about 100 infrared light pulses separated by the round-trip transit time of light in the laser cavity. Each pulse contains about 1 millijoule of energy at 1.06 micrometers and has a full width at half maximum of 5 to 10 psec.

For most experiments a single pulse is selected from the train and amplified to ~ 50 millijoules. The infrared pulse may be used directly to irradiate the sample or may be frequency-shifted by various nonlinear techniques such as harmonic generation or stimulated Raman scattering to produce excitation at other wavelengths. In addition, various probe pulses or shuttering pulses, or both, are also derived from the fundamental pulse. Calibrated delays between the initiation pulse and probe pulses provide time resolution of the processes occurring in the sample.

Single-pulse measurements are preferable to those in which many pulses are used, regardless of whether the pulses are from the same or different pulse trains, since pulse characteristics such as energy, time and frequency widths, and the pulse substructure vary not only from one train to the next but within a single train as well. For these reasons we usually employ the echelon technique (*5*) (stepped optical delay) whereby it is possible to record the complete time infor-

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